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Evaluating the Impacts of Biochar on the Fate and Dynamics of Dairy Manure in Agricultural Soil

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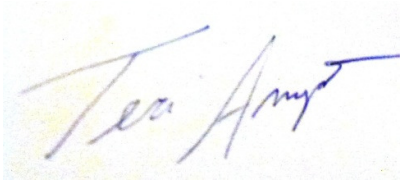
Declaration

I declare that this thesis has been composed by myself and has not been submitted in any previous application for a degree. The work described is my own except where stated otherwise.



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Abstract

Biochar is a carbon sequestration technology that has shown potential to inhibit greenhouse gas (GHG) emission and nutrient leaching from soils, however the majority of biochar research thus far has focused on arable cropland rather than livestock systems or grasslands. Livestock production is an important agricultural system, and manure generated from livestock systems is a source of GHG emission as well as nutrient loading to surface- and groundwater. The high environmental impact of livestock production in the very areas that biochar has shown potential may suggest that this would be an ideal system for biochar incorporation. However, as grassland systems in the context of livestock production often receive high nutrient inputs in the form of manure which increases the potential for nutrient leaching or runoff, the high-nutrient ash content of biochar may potentially exacerbate this problem rather than suppress nutrient loss from soils. As private companies and government-funded programmes discuss the possibility of scaling the global manufacturing and soil-incorporation of biochar to a rate of gigatonnes per year, understanding the potential of biochar for use within a livestock system could be crucial in helping to develop an appropriate deployment plan for this material.

This thesis is therefore focused on the use of biochar in grassland and livestock systems. It first examines the nutrient release from biochar in a sequential leaching experiment. Phosphorus (P) release indicated that provision of soil P (though quantitatively small) may be sustained over time whilst potassium (K^+) release was quantitatively large but declined rapidly following the first extraction. An incubation study was then carried out using soil columns amended with farmyard manure, liquid manure (slurry) or fertiliser (plus an unamended control), each with and without biochar, which sought to determine the impact of biochar on N_2O release and N and P leaching from soils with diverse nutrient sources. N_2O emission from the columns was significantly suppressed by the presence of biochar, as was the leaching of mineral N. However, the amount of PO_4^{3-}-P in leachate was increased in biochar-amended columns, relative to their unamended counter-parts. A slurry incubation study was then conducted, with a control slurry and four biochar-amended treatments, which explored whether biochar could suppress GHG and NH_3 emission from manure

prior to land application. The resulting data indicated that biochar demonstrates potential for GHG suppression but does not demonstrate potential for NH_3 suppression from slurry in storage. Finally, a one-year field-based experiment was completed which analysed the impact of biochar on CH_4, N_2O, and NH_3 emission as well as nutrient leaching from grassland soils that had been amended with a high rate of manure application (151.4 m^3 ha^{-1} or 409 kg N ha^{-1}). In this study, biochar demonstrated the potential to suppress each of the three types of gaseous emissions from manure-amended soil, though the differences between mean values were not statistically significant. Extracts from ion exchange resins indicated that annual cumulative K^+ leached from biochar-amended plots was significantly higher than the control, and that P and NH_4^+ leached from biochar-amended plots was higher than the control at the time of the first rain event following biochar and manure application. Together, the results of these component studies indicate that biochar may indeed have potential to suppress GHG emissions from livestock systems, most likely through suppression of microbial activity by organic compounds that are sorbed to the char, though (as the mechanisms of GHG suppression by biochar are thus far not well understood) the capacity of biochar to do so may vary based on the type of biochar used, the soil characteristics, and other factors. Overall, the results of these studies suggest that some types of biochar should be used with caution in systems with high rates of nutrient application, unless the ash is removed prior to soil application.

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Contents

Chapter 1

Introduction

1.1 Livestock systems and effects on the environment

Livestock production is an important agricultural system which accounts for 70% of all agricultural land and 30% of the land surface of the planet. It accounts for 40% of worldwide agricultural gross domestic product and provides one third of human protein intake (FAO, 2006). Furthermore, due to the steady increase in global population and the shift of dietary preferences to higher value foods such as meat and milk, livestock production has rapidly increased in recent decades. Global production of meat is expected to more than double and milk production is expected to increase by more than a third by 2050 (FAO, 2006; Popp et al., 2010).

In the United Kingdom, dairy cows are housed throughout the year in some systems and grazed throughout the year in others (Haskell et al., 2007) whilst beef cattle consist mainly of culled dairy cows that are largely raised indoors and specialised beef production systems in which the cows graze outdoors in the summer and stay indoors in the winter (Nguyen et al., 2010). Although cattle were traditionally left to graze for most of the year, there is a trend toward increased intensification with longer housing periods (Haskell et al., 2003), though public opinion of animal welfare may have an effect in curtailing this trend (Ellis et al., 2009) and silage costs and environmental problems created by silage effluent have encouraged grazing outside the normal season (Laidlaw and Mayne, 2000). In the European Union, the Nitrates Directive requires individual countries to monitor water quality and to create action plans to improve water quality, generally by limiting the rates and timing of fertiliser and manure application, defining manure storage capacity, and limiting animal grazing (Brouwer et al., 2002). Manure stores within nitrate vulnerable zones (areas of land that drain into waters polluted by nitrates) are subject to strict regulation (DEFRA, 2009).

Although grazing through much of the year is more common in the U.K., intensive housing systems are more common in the U.S. (Mee, 2012). In areas like California that have low rainfall throughout much of the year, careful range management and housing of

cows throughout the summer dry season are necessary (McDougald et al., 2001). Widespread overstocking during the California gold rush followed by periods of drought and flooding during the late 19th and early 20th centuries deteriorated the grazing lands and forced livestock owners to learn how to utilise grazing lands in a sustainable manner (CCWD, 2005). In the U.S., confined animal operations in a watershed that is being monitored under the Clean Water Act (CWA) may be considered a point source of pollution and therefore would be required to meet manure storage, handling, and disposal requirements under the CWA. However, precipitation-related discharges from these operations are exempt from CWA discharge rules if compliance with Nutrient Management Technical Standards (NMTS) can be demonstrated (Federal Register, 2003). For livestock operations that are not being monitored under the CWA, the Federal Rule which addresses manure storage only requires that there is adequate manure storage volume for the facility to process water, rain events, and manure (Federal Register, 2003).

Livestock manure from housed cattle (when mixed with the straw from bedding) can be used as a soil amendment in agriculture. Cattle manure and slurry are common organic fertilisers applied to grasslands and arable cropland, due to their high nitrogen supply and rich composition of nutrients. Cattle farmyard manure contains 6.0 kg total nitrogen (N) t^{-1} (fresh weight) whilst cattle slurries contain 1.6-3.6 kg total N m^{-3} (fresh weight) (DEFRA, 2010). Nitrogen can be present in manure as ammonium-N and uric acid-N, which are potentially available for rapid crop uptake, or organic-N, most of which is not available in the first season after application as organic-N mineralises slowly over months to years (DEFRA, 2010). Slurries are relatively high in readily available-N (40-60% of total N). The major N loss processes following land application include ammonia (NH_3) volatilisation, nitrate (NO_3^-) leaching, and denitrification (DEFRA, 2010).

Manures are valuable sources for other nutrients as well, though the total nutrient content is not available for the first season following application as they are slowly mineralised over months to years (DEFRA, 2010). Cattle farmyard manure contains 1.5 kg t^{-1} phosphorous (P) and 6.6 kg t^{-1} potassium (K^+) on a fresh weight basis. However, the

availability of manure P the first season is approximately 60%, which is lower than water soluble P fertiliser (DEFRA, 2010). On the other hand, around 90% of manure K^+ is readily available for plant uptake in the first season. Cattle slurries contain 0.3-0.9 kg m^{-3} total P (50% of which is readily available) and 1.2-4.2 kg m^{-3} total K^+ (90% of which is readily available) (DEFRA, 2010).

Manure and slurry are often stored for a period of months prior to land application due to inconvenience of land application or constraints to field access, including seasonally wet soil. Additionally, storage of manure can allow application at times of highest crop growth or lowest rainfall in order to facilitate higher crop uptake of manure nutrients. Utilisation of manure from confined livestock shelters is a growing problem, particularly with large-scale operations in which availability of land for manure application is limited (Power and Schepers, 1989; Sharpley et al., 1998). Despite the nutrient content of manures, land application of manure is often considered primarily a waste management practice in dairy systems. Due to the low density of nutrient value in manure compared to commercial fertilisers, the cost of transport more than a short distance exceeds the nutrient value of the manure. Thus, the land area to which the manure can reasonably be added is limited to a short radius from the point of production (Sharpley et al., 1994; DEFRA, 2004), which frequently includes fields in which grasses are grown for silage to feed cattle in seasons and environments in which grazing is not feasible. Nutrient loading in surface and ground water is directly related to stocking densities, as the addition of manure nutrients to soil in excess of that which the crops can use can result in nutrient leaching, which is an important source of water pollution (Stout et al., 2000; Buda and Klelnman, 2009). Furthermore, if manure is applied at a rate sufficient for crop N requirements, accumulation of soil P is inevitable, which is likely to result in P leaching and runoff (Olson et al., 2010). Importantly, manure and slurry are a source of NH_3 emissions as well as greenhouse gas (GHG) emissions such as methane (CH_4), nitrous oxide (N_2O) and carbon dioxide (CO_2) (Flessa and Beese, 2000; Rodhe et al., 2006).

1.1.1 Nutrient leaching

N (as NO_3^-) in particular is mobile in many soils and often leaches or volatilises (as NH_3) to the atmosphere, re-depositing elsewhere (Carpenter et al., 1998). On a typical dairy farm 17% of N loss is through leaching (DEFRA, 2004). For efficient use of N, manures should be applied to fields at times of maximum plant growth. NO_3^- leaching is avoided by careful adjustment of the application rate, and avoidance of application during periods of heavy rainfall (DEFRA, 2010). High levels of nitrogen may damage ecological systems (Vitousek, 1997) and contaminate groundwater (Spalding and Exner, 1993). In California, where the number of cows per farm is increasing and farms are highly concentrated in specific geographic areas, NO_3^- contamination has been declared by the Lawrence Livermore National Laboratory to be 'the number one contaminant threat to California's drinking water supply' (Moore et al., 2011). N cycling in livestock systems is illustrated in Figure 1.

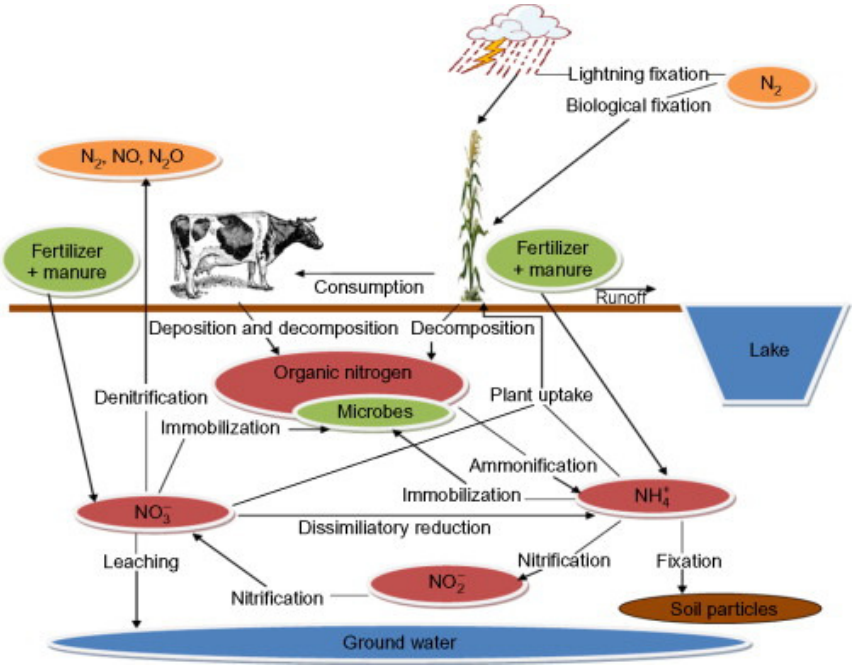

Figure 1: Illustration of the nitrogen cycle in soil
(St. Luce et al., 2011)

P loss from manure application can be reduced by monitoring soil P levels and avoiding over-application, as well as by avoiding surface applications of manure to steeply sloping land (DEFRA, 2010). There is a linear relationship between total soil P and solubilised

P in leachate (DEFRA, 2004). P accumulation in soils in the UK has been recorded at rates as high as 26 kg ha^{-1} y^{-1} and P loss from agricultural systems as high as 17 kg ha^{-1} y^{-1} (Sharpley et al., 1994; DEFRA, 2004). Freshwater systems are highly sensitive to pollution by P (Carpenter and Bennett, 2011), which is a limiting nutrient for algal growth and eutrophication (Carpenter, 2008; Schindler et al., 2008). Although eutrophication can cause damage that can be costly (Environment Agency, 2002), a second cost of P leaching to farmers is import of fertiliser which is a finite resource (Cordell et al., 2009). Central to managing the agricultural P cycle is the effective recycling of manure P (Bateman et al., 2011).

As with P, soils can be overloaded with potassium (K^+) when manure is applied for optimum N input, though K^+ loss to surface- and ground-water receives less attention than P, as K^+ is generally not considered to be a pollutant. However, K^+ is highly soluble in water and K^+ loss via leaching (particularly from sandy soils) can be as high as 20-50 kg ha^{-1} $year^{-1}$ (Askegaard et al., 2004). Furthermore, in systems with soils or irrigation water that have a high calcium (Ca) content, the Ca^{2+} cation is the dominant cation and competes for exchange sites with K^+, leading to an increase in K^+ leaching from soils (Jalali and Rowell, 2009). As crop K^+ requirements in agriculture are second only to N, effective K^+ management is important (Askegaard et al., 2004).

1.1.2 NH_3 production

Meat and milk production are inefficient with respect to N usage, as 70-95% of the N that is consumed by livestock is excreted, and approx. 25% of the N in this system is lost to the atmosphere as NH_3 (Ferm, 1998; Oenema et al., 2005), decreasing the fertiliser value of manure. NH_3 can also be oxidised and partly transformed to N_2O (Ferm, 1998). Furthermore, NH_3 deposited in natural ecosystems may cause soil acidification (van Breemen et al., 1982) and degradation of ecosystems (Bäck et al., 1997). In order to prevent NH_3 volatilisation, manures and slurries should be rapidly incorporated into soils, and injection or band spreading techniques can also be used for slurries (DEFRA, 2010). Additionally, as a large portion of NH_3 emissions emanate from stables and manure

storages (Ferm, 1998; Misselbrook et al., 2000), further treatment can be undertaken to reduce NH_3 emission prior to land application through the use of additives (McCrory and Hobbs, 2001) or physical covers or barriers (Petersen et al., 2009).

1.1.3 GHG emission

The enormous scale of worldwide livestock production has impacted the environment in many ways, not the least of which is through GHG emission, which is emitted from livestock manure and contributes to climate change and (in the case of N_2O) to ozone depletion (IPCC, 2007; Skiba et al., 2012). Manure management is important for GHG emission control and treatment systems have demonstrated the potential to significantly mitigate gaseous emissions (Oenema et al., 2007; Aneja et al., 2008).

CO_2 is released from livestock manure following anaerobic bacterial degradation of manure organic matter as well as aerobic microbial degradation at the slurry-air interface (Patni and Jui, 1985; Steed and Hashimoto, 1994; Møller et al., 2004). In dairy systems, CO_2 release receives less attention than other GHGs, as the CO_2 produced by animal manure was previously absorbed from the air via plants providing feed for the animal, and thus is considered a part of carbon cycling rather than a net GHG source (Külling et al., 2002). Furthermore, emission of CH_4 and N_2O to the atmosphere (though lower in magnitude than CO_2) have much higher global warming potentials (GWP) than CO_2 (IPCC, 2001).

Atmospheric concentrations of CH_4 are an order of magnitude lower than CO_2, but the GWP is 25 times higher over a 100 year time horizon (IPCC, 2007). The effects of hydrocarbons on the degradation of the ozone layer have also led to increased concern around CH_4 from livestock systems (Hill et al., 2001). Enteric fermentation is estimated to account for approx. 85% of all CH_4 emission in agriculture, and emissions from slurry stores, manure handling, and manure spreading account for the majority of the remainder (Monteny et al., 2006). CH_4 from manure is produced by microbes as a by-product of digestion of structural carbohydrates (Hopkins and Del Prado, 2007) and thus is

dependent on the diet of the ruminants (Külling et al., 2002), manure total solids content, and pH (Petersen et al., 2012; Wood et al., 2012), among other factors. Stored manure with sufficient dry matter content that is not mixed or disturbed will form a dense surface crust of dried organic fibres and bedding material (Sommer and Hutchings, 1995). Such crusts have been demonstrated to reduce CH_4 emission (Husted, 1994; Sommer et al., 2000) and to increase CH_4 oxidation (Petersen et al., 2005). In addition to emission from manure storage, intensive slurry application to soil can produce substantial amounts of CH_4 (Chadwick and Pain, 1997; Chadwick et al., 2000; Rodhe et al., 2006), despite the fact that soils have been demonstrated to be an important sink for CH_4 (Boeckx and van Cleemput, 2001).

N_2O formation in soil occurs through three key biological mechanisms: aerobic autotrophic nitrification, anaerobic heterotrophic denitrification, and nitrifier denitrification. Nitrification is more likely to predominate in dry conditions and in coarse textured soils while denitrification (which is the main mechanism of N_2O production in agricultural soils) often predominates in wet conditions and fine textured soils, though both processes can occur simultaneously (Wrage et al., 2005; Opdyke et al., 2009; Senbayram et al., 2009). N_2O has an atmospheric lifetime of 114 years, a GWP of 298 over a 100-year time horizon (Solomon et al., 2007), and is the single most important contributor to stratospheric ozone depletion (Ravishankara et al., 2009). It has been estimated that 2.0% of manure N has been emitted to the atmosphere as N_2O since 1860 (Davidson, 2009), and N_2O fluxes from manure-amended soil can be greater in magnitude and extend over a longer period of time than mineral fertiliser (Jones et al., 2007; Senbayram et al., 2009). N_2O emissions are often greater in grasslands than arable agriculture due to soil compaction and higher N inputs (Velthof et al., 1996). High manure inputs in intensive livestock systems have also increased N availability and thereby greatly enhanced N_2O emissions from soils (Kroeze et al., 1999). Furthermore, N_2O emissions from stables and storages are nearly double those arising from soil applied waste (Oenema et al., 2005). Although N_2O is not typically produced in slurries, it may be produced during long periods of storage if a surface crust forms (Wood et al., 2012).

1.2 Biochar effects on nutrient retention and gaseous emissions

Biochar is defined as a carbon-rich product obtained when biomass is heated in a closed chamber with little or no available air, driving off volatile gases (Lehmann and Joseph, 2009). Biochar has been proven to be more stable than any other amendment to soil, and to increase nutrient availability (Chan and Xu, 2009; Lehmann, 2009). Pre-Columbian Amazonians are believed to have use biochar to increase the productivity of soils, and Europeans settlers called the resulting carbon-rich soil Terra Preta de Indio (Glaser et al., 2001). Due to its high C content and stability, biochar has been proposed as a means of storing C in the soil (Lehmann et al., 2006) (Figure 2).

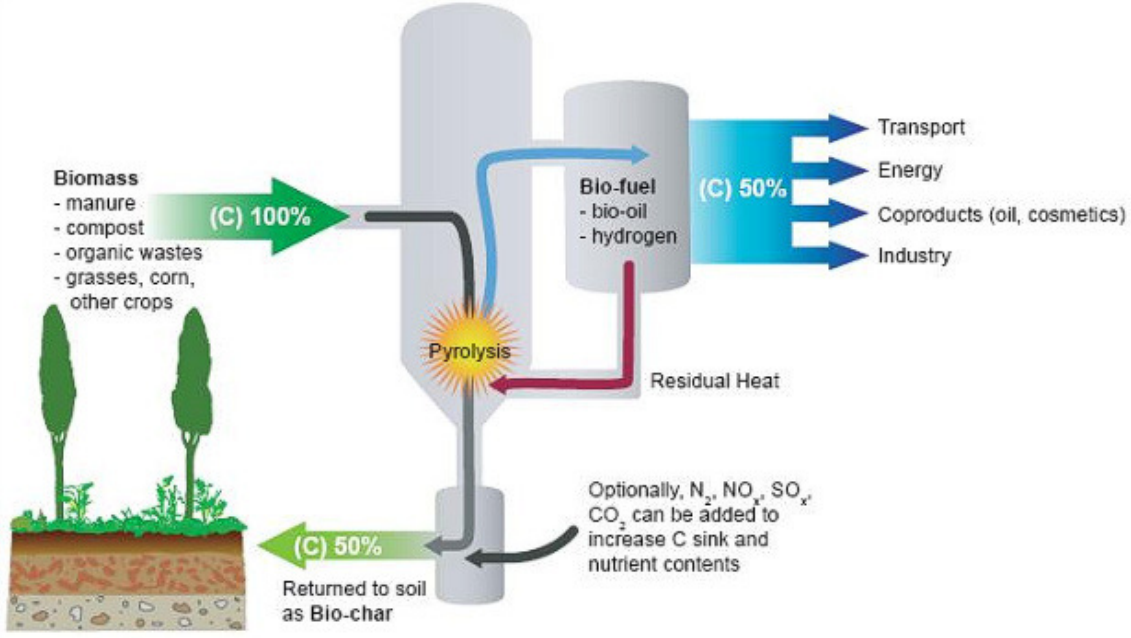

Figure 2: Concept diagram of pyrolysis with biochar sequestration
(Source: Johannes Lehmann)

Furthermore, turning organic matter inputs into biochar can decrease CO_2 emission by considerably lowering its rate of decomposition in soil (Sohi et al., 2010). Biochar and charcoal are often used interchangeably for the purposes of scientific research, as biochar is primarily distinguished from charcoal and similar materials by the fact that it is

produced with the intent of application to soils rather than for use as a fuel source (Lehmann and Joseph, 2009).

Biochar can have a strong influence on the physical and chemical properties of soil. The presence of biochar alters the physical makeup of soil horizons, thereby affecting the response of soil to water. The addition of biochar can improve filtration of percolating soil water, improve aeration in a clay soil or improve water holding capacity in a sandy soil (Downie et al., 2009; Lehmann and Joseph, 2009). Depending on the feedstock material and methods used in producing the biochar, the ash content of the biochar can directly supply nutrients to plants and biochar can also retain nutrients in the soil, thereby increasing nutrient savings and fertiliser use efficiency (Lehmann et al., 2003; Chan and Xu, 2009). The high surface area of biochar can also improve nitrogen fertiliser retention and reduce leaching (Lehmann et al., 2003).

1.2.1 Effect of biochar on nutrient retention

In addition to the direct nutrient value of biochar, many of the positive crop responses to biochar application have been attributed to indirect effects on soil nutrient properties, such as the sorption of nutrients. The ability of biochar to retain applied fertiliser against leaching has been related to the charge in surface area properties of biochar (Chan and Xu, 2009).

It has been found that wood charcoal can be effective in the removal of ammonium (NH_4^+) from aqueous solutions (Asada et al., 2006) and as an adsorbent of NO_3^-, nitrite (NO_2^-), and phosphate (PO_4^{3-}) (Fujita et al., 1991). Biochar derived from sugar beet tailings has also proven more effective than activated charcoal in the removal of PO_4^{3-} from aqueous solution (Yao et al., 2011), and biochar derived from anaerobically digested fibre has demonstrated potential to decrease P in dairy lagoons due to the adsorption of PO_4^{3-} ions to biochar particles used in a filtration system (Streubel et al., 2012).

Nutrient retention studies that have described the incorporation of biochar into soil have been mainly incubation, column, or pot experiments. Most nutrient retention studies incorporated wood-derived biochar though some studies utilised biochar made from poultry litter, pecan shell, switch grass, wheat straw, or sugarcane bagasse. Application rates have ranged from approx. 4.8 t ha^{-1} to 644 t ha^{-1} (Laird et al., 2010; Doydora et al., 2011).

In comparison with other plant nutrients, biochar has demonstrated the highest potential in the retention of NH_4^+, as many incubation studies reported that NH_4^+ concentrations in leachate decreased following biochar application (Lehmann et al., 2002; Lehmann et al., 2003; Ding et al., 2010). Singh et al. (2010) reported a decrease in NH_4^+ from biochar-amended soils following the second leaching event, though this was not consistent between the two soil types that were used. All of these studies cited adsorption to the biochar surfaces as the likely mechanism, and Singh et al. (2010) suggested that the biochar had aged between the first and second leaching events, thereby increasing the cation exchange capacity (CEC) and the capacity for the biochar to adsorb the NH_4^+. Only one experiment reported an increase in NH_4^+ following (fast pyrolysis) biochar addition (Bruun et al., 2012), attributed to suppressed nitrification, as this study also reported decreased NO_3^- loss in leachate from soils amended with the same type of biochar. A decrease in NO_3^- in leachate has been reported by others (Knowles et al., 2011; Ippolito et al., 2012), and has been attributed to immobilisation following the addition of C. However, an increase in NO_3^- in leachate following biochar addition has also been reported (Lehmann et al., 2003; Singh et al., 2010). Additionally, some studies have reported mixed results. For example, Laird et al. (2010) found that biochar increased NO_3^- in leachate from soils amended with only biochar (relative to the control), but that biochar decreased NO_3^- in leachate from soils amended with both biochar and dried swine manure (relative to soils amended with only swine manure). It has been stated that (in these cases) it is unlikely that NO_3^- has been adsorbed to the biochar, or alternately that it may be weakly adsorbed and easily desorbed (Ippolito et al., 2012; Kameyama et al., 2012), and therefore it is likely that suppression of NO_3^- loss via leachate is due to other

mechanisms. Studies that have measured total N in leachate have reported a wide range of results, including suppression of N loss from biochar amended soils (Steiner et al., 2009), an increase in N in leachate following a suppression of NH_3 volatisation (Doydora et al., 2011) and no significant difference between treatments due to the use of a biochar with a relatively small surface area (Bruun et al., 2012).

Incubation studies that have measured P in leachate have also reported conflicting results. Those who have observed a decrease in P loss in leachate from biochar amended soils (Novak et al., 2009; Laird et al., 2010) have attributed this to the adsorption of orthophosphate and organic P to the surface of the biochar. Those who have observed an increase in P loss in leachate (Ippolito et al., 2012; Kameyama et al., 2012) have theorised that P content in biochar that was chosen for these experiments was in a particularly soluble form.

In contrast, the effect of biochar on K^+ leaching has been reported to be relatively consistent between studies. The presence of biochar in soils has generally increased K^+ in leachate (Lehmann et al., 2003; Novak et al., 2009; Steiner et al., 2009; Laird et al., 2010; Ippolito et al., 2012; Sarkhot et al., 2012). This has been attributed to the high K^+ content of the biochar, the high solubility of K^+, and the fact that biochar exchange sites are preferentially occupied by Ca^{2+}, Mg^{2+}, and other multivalent cations. However, a mass balance analysis (Laird et al., 2010) indicated that the presence of biochar could decrease leaching of manure K^+, and that the combined effects of multiple sources of K^+ (from manure, soil, and biochar) influenced the mobility of K^+ in soil and leachate.

Only one study has measured nutrient leaching in the field after biochar addition using suction lysimeters (Major et al., 2012) in an acidic clay soil cultivated under a maize-soybean rotation. Although increases in soil solution concentrations of NO_3^-, NH_4^+, and K^+ were observed in the root zone of amended soils, decreases were observed in soil solution at 1.2 m (NO_3^- and K^+) or 2.0 m (NH_4^+) depths. Significant decreases in P soil solution concentrations were observed at both the 0.3 m depth and the 2.0 m depth. These

results highlight that the impact of biochar on the movement of nutrients through the soil and the retention of solutes in the soil is complex. The lower amounts of nutrients below the rooting depth suggest a differential uptake by plants at different times.

1.2.2 Effect of biochar on gaseous emissions

It has been noted that biochar application can reduce GHG emission from soil (Lehmann et al., 2006; van Zwieten et al., 2009; Singh et al., 2010). The majority of studies measuring GHG emission from soil amended with biochar have been incubation studies, though there have been a few field-based studies (Karhu et al., 2011; Scheer et al., 2011; Taghizadeh-Toosi et al., 2011; Wang et al., 2011; Liu et al., 2012; Zhang et al., 2012). A range of soil and biochar types have been used, and soils have been selected from rice paddies, almond orchards, and pasture fields, as well as fields used to grow corn, soybeans, wheat, and oats. Many of these studies have incorporated commercial fertilisers or (in the case of pasture soils) bovine urine and urea. Anaerobic digester slurry, dried swine manure, dried sewage sludge, and compost have also been used as the associated source of N (Bruun et al., 2011; Rogovska et al., 2011; Aguilar-Chávez et al., 2012; Kammann et al., 2012). The application of farm yard manure and dairy slurry to fields used to grow grasses for silage has not been addressed in this context.

Most biochar application incubation studies have suggested favourable results in terms of the suppression of N_2O emission from soil (Yanai et al., 2007; Clough et al., 2008; Rogovska et al., 2008; Sohi et al., 2010; van Zwieten et al., 2010; Rogovska et al., 2011; Aguilar-Chávez et al., 2012; Case et al., 2012; Jia et al., 2012; Sarkhot et al., 2012). However, in studies where more than one biochar type and/or soil type have been used, results were not consistent between biochar types (Spokas and Reicosky, 2009; van Zwieten et al., 2009; Singh et al., 2010; Kammann et al., 2012; Yoo and Kang, 2012). However, for the majority of biochar types, N_2O emissions were suppressed relative to the control. Relatively few studies found no effect of biochar or an increase above that of the control (Clough et al., 2010; Bruun et al., 2011). Many authors have noted that cases where N_2O was not suppressed either included a high N (fertiliser) input or biochar

with a high indigenous N content (Spokas and Reicosky, 2009; van Zwieten et al., 2009; Singh et al., 2010). In support of this view, Wang et al. (2011) found that biochar decreased N_2O emission from control soils, but increased N_2O emission from fertiliser-amended samples in one of two incubation studies in comparison to soils that had only been amended with fertiliser but not biochar. Occasionally, N_2O suppression is only reported in cases of very high biochar application rates (Spokas et al., 2009). Indeed, application rates in some incubation studies that were successful in mitigating N_2O emission were the equivalent of 180, 720, or 644 t ha^{-1} (Yanai et al., 2007; Spokas et al., 2009; Rogovska et al., 2011, respectively). The rate of application used by Rogovska et al. (2011) was on a dry weight basis; the rate of application used in the other two studies was not specified as dry weight or fresh weight.

Field-based studies have been fewer in number, and with more modest results. Rice paddy studies incorporating wheat straw-based biochar at a rate of 20 t ha^{-1} have been successful in reducing N_2O emission over that of the control (Liu et al., 2012; Zhang et al., 2012). However, birch-based biochar incorporated into an organic crop rotation and cattle feedlot biochar incorporated into a peanut and ryegrass rotation at rates of 9 and 10 t ha^{-1} (respectively) have reportedly had no significant impact on N_2O emission (Karhu et al., 2011; Scheer et al., 2011). In contrast to results from the incubation study by Wang et al. (2011), Taghizadeh-Toosi et al. (2011) found that plots where biochar was incorporated into soil at a rate of 10, 20, or 30 t ha^{-1} emitted more N_2O than control plots, but that soils amended with both 30 t ha^{-1} biochar and urea emitted less N_2O than plots amended with only urea.

Many authors attribute N_2O suppression to changes in physical characteristics of the soil, such as bulk density, aeration, and pore size distribution (Yanai et al., 2007; Clough et al., 2008; Sohi et al., 2010; Rogovska et al., 2011), though Case et al. (2012) rejected soil aeration as a mechanism in N_2O suppression through biochar addition. Some studies observed a suppression of nitrification (Clough et al., 2010) or a suppression of denitrification (Clough et al., 2008) following biochar addition. Some authors attribute

suppression of denitrification to the increase in pH (van Zwieten et al., 2010) due to the liming effect of the ash content of the biochar, though others have rejected this as a mechanism (Yanai et al., 2007; Case et al., 2012). Some have suggested that NH_4^+ and NH_3 are sorbed to the biochar, making this N unavailable for nitrifying bacteria, though it has also been suggested that the NH_4^+ can be desorbed later (Clough et al., 2010; Taghizadeh-Toosi et al., 2011; Wang et al., 2011). It has also been suggested that more soil N is assimilated into microbial biomass on account of the high C-to-N ratio of the biochar addition (Wang et al., 2011; Case et al., 2012), however the labile fraction is usually much less than 1% of the total C in biochar (Zimmerman, 2010; Cross and Sohi, 2011) and is not likely to have a great impact at low rates. Several studies in which N_2O has not been suppressed have attributed this result to high concentration of available N and an abundance of pre-existing denitrifiers (Yoo and Kang, 2012), low biochar application rates (Karhu et al., 2011), or an ineffectual combination of biochar and soil type (Scheer et al., 2011).

Results from biochar studies that have monitored CH_4 or CO_2 emission have been even less conclusive than the N_2O studies. Some incubation studies have indicated no treatment effect on CH_4 emission (Aguilar-Chávez et al., 2012; Jia et al., 2012). Others found a suppression of net CH_4 emission from pasture and rice paddy soils (Feng et al., 2012; Liu et al., 2011; Yoo and Kang, 2012), or a suppression of soil methanotrophic activity in a soil incubation resulting in increased net CH_4 emission (Spokas and Reicosky, 2009). Field experiments have indicated both no significant effect from soils used to grow wheat or peanut and ryegrass (Castaldi et al., 2011; Scheer et al., 2011) and an increase in CH_4 emission from rice paddy soils (Zhang et al., 2012). All authors of papers in which there were significant treatment effects attributed these to an inhibition of microbial activity either by suppression of methanogens or suppression of methanotrophs. It was also suggested that CH_4 suppression could be due to lack of substrate availability or direct absorption of CH_4 gas (Liu et al., 2011; Yoo and Kang, 2012).

Likewise, incubation studies have indicated inconsistent effects on CO_2 emission between biochar or soil types within the same study, or between experiments within the same paper (Spokas and Reicosky, 2009; Wang et al., 2011; Kammann et al., 2012). Biochar addition in some incubation studies resulted in an increase in CO_2 emission (Bruun et al., 2011; Rogovska et al., 2011), others have resulted in a decrease in CO_2 emission (Spokas et al., 2009; Aguilar-Chávez et al., 2012; Sarkhot et al., 2012), and still others have resulted in no significant treatment effect (Liu et al., 2011; Yoo and Kang, 2012). Field experiments have indicated no effect of biochar addition on soil respiration (Castaldi et al., 2011; Karhu et al., 2011; Zhang et al., 2012). Experiments for which there was no treatment effect suggested that this indicated that biochar is recalcitrant and that there is no priming effect (Zhang et al., 2012). Decreases in CO_2 emission have been attributed to the lack of available N due to the increase in soil C-to-N ratio following biochar addition (Liu et al., 2011), and increases have been attributed to the soil microbial community having been C-limited (Bruun et al., 2011) or limited in micronutrients in cases in which was no fertiliser addition (Spokas and Reicosky, 2009). The variation of results has been attributed to soil and biochar type, suggesting that any losses of CO_2 are probably short-lived, as the labile carbon content of the biochar is limited (Wang et al., 2011; Sarkhot et al., 2012).

Suppression of all GHG emissions (and, indeed, increases in CH_4) have been attributed to a suppression of microbial activity. It has been suggested that organic substances that are adsorbed to the biochar are toxic to the microbial communities. This could explain the need for high application rates to induce significant emissions reductions, as well as (in some cases) the suppression of methanotrophic bacteria (Spokas and Reicosky, 2009; Bruun et al., 2011; Taghizadeh-Toosi et al., 2011).

Biochar has been found to absorb NH_3 or suppress NH_3 emission from composting chicken manure (Clough and Condron, 2010; Steiner et al., 2010; Spokas et al., 2011), but there have been very few studies regarding the impact of biochar on NH_3 emission from soil, and no studies regarding the impact on NH_3 emission from cattle manure in

storage. However, two studies published by Clough et al. (2008; 2010) indicated no treatment effect following biochar addition, and that biochar increased NH_3 emission, respectively. Schromberg et al. (2012) reported that biochar increased NH_3 emission from soil due to the increase in soil pH. Conversely, Taghizadeh-Toosi et al. (2012) reported that biochar suppressed NH_3 emission from field soils amended with biochar.

1.3 Context of thesis

As Belize and several African government agencies have recommended that biochar be recognised as a climate change mitigation and adaptation technology (Harrabin, 2009), and as there are growing numbers of private companies interested in the manufacture and distribution of biochar, there exists the possibility that biochar could be scaled to megatonnes of production and soil incorporation annually in some regions of the world, particularly if carbon credits are eventually developed and deployed. As 70% of all agricultural land (and, indeed one-third of the land surface of the planet) is dedicated to livestock and as the livestock sector could benefit from technologies that abate GHG emission and nutrient leaching (FAO, 2006), understanding the potential of biochar for use within a livestock system could be crucial in helping to develop an appropriate deployment plan for this material. This thesis is therefore focused on the use of biochar in grassland and livestock systems. Wood-derived biochar has been chosen for use in these studies as wood is currently the prevalent feedstock material for the manufacturing of biochar in both research and industry.

UK and northern California dairy farm environments were the focus of these studies due to the presence of biochar producers and interest in these areas of the world. Dairy farmers in northern California in particular have expressed an enthusiasm for adoption of practices that bring a premium price for milk and allow for them to stay competitive against large-scale factory farming organisations. Farmers in this area have embraced organic and sustainability practices as well as certification procedures demonstrating the ethical treatment of cows, and have demonstrated an interest in learning about practices that could allow for new ways of marketing their products. For instance, if the carbon footprint (Wright et al., 2011) of a product indicates that the net GHG emission generated in the development and distribution of a product are less than the amount of carbon sequestered by biochar application to fields associated with the product, it may be possible for a farmer to market the product as ‘carbon negative’. Carbon negative products have been explored in other industries, such as rubber latex (Tilakaratne 2011).

The vast majority of biochar research to date has been conducted with the use of arable cropland soils, and very little work has been done utilising the combination of manure and biochar, following (or even prior to) addition to soil. Furthermore, biochar research that has been conducted on grassland soils has focused on perennial grasses rather than annual grasses harvested for silage (Taghizadeh-Toosi et al., 2011; Kammann et al., 2012). Due to its demonstrated ability to impact gaseous emissions from soils (Lehmann et al., 2006; van Zwieten et al., 2009; Singh et al., 2010), the addition of biochar to manure could potentially begin affecting GHG emissions even before application to the soil. Moreover, most existing biochar research has consisted of laboratory and incubation studies. Thus, this thesis includes a biochar nutrient release study and two soil incubation studies, one of which includes a comparison of the effect of biochar on farmyard manure with the effect of biochar on fresh (undigested) cattle manure slurry, which has not been addressed in prior literature. Additionally, this thesis includes a study that addresses the impact of biochar on liquid manure in storage which has not been assessed in prior literature. Finally, given the dearth of biochar field-based experiment data, this thesis concludes with a one-year field-based study. This study was conducted in a field that grows annual ryegrasses for silage (which has not yet been used in biochar research) and includes an assessment of the impact of biochar on nutrient loss from manure-amended soil via leaching which had not yet been addressed in the field environment. These studies contribute to the existing knowledge and understanding of the use of biochar in agricultural systems.

1.4 Thesis aims and objectives

The aim of this thesis is to improve understanding of the use of biochar in a livestock or grassland system, particularly the impact of biochar on nutrient retention and GHG emission from soil amended with manure or slurry as well as the impact of biochar on GHG emission from slurry in storage. As there is a dearth of data from field-scale biochar experiments, a second aim of this thesis is to contribute a year of data on the field scale following biochar application to a ryegrass field. The hypotheses that are addressed in this thesis include:

- Biochar incorporated into sandy soils used to grow ryegrasses for silage will decrease nutrient leaching following manure application.
- Biochar incorporated into sandy soils used to grow ryegrasses for silage will decrease GHG and NH_3 emission following manure application.
- Biochar incorporated into slurry that is in storage will decrease slurry GHG and NH_3 emission.
- Biochar applied as a cap to slurry that is in storage will decrease slurry GHG and NH_3 emission.

This thesis is structured as a series of papers intended for publication, with the main aims and summary of each paper as follows:

1.4.1 Chapter 2 (Paper 1): Establishing release dynamics for plant nutrients from biochar

As one aim of this thesis is to understand the impact of biochar on nutrient retention in agricultural soil, the release patterns and direct supply of crop nutrients from fresh biochar were first considered. The main aim of this paper was:

- To assess the magnitude and dynamics of short-term P, Mg, and K release from a hardwood biochar at different particle size ranges using two repeated cold water extraction techniques.

1.4.2 Chapter 3 (Paper 2): Biochar diminishes nitrous oxide and nitrate leaching from diverse nutrient sources

Prior to conducting a field-based study, a 55 day soil column incubation study was conducted. The main aims of this paper were:

- To test the hypothesis that biochar decreases N_2O emissions from soil amended with mineral fertiliser, manure, or slurry.

- To test the hypothesis that biochar decreases nutrient leaching from soil amended with mineral fertiliser, manure, or slurry.

1.4.3 Chapter 4 (Paper 3): The impact of biochar on gaseous emissions from liquid dairy manure

In order to assess the impact of biochar on liquid dairy manure in storage, a 107 day manure incubation study was conducted. The main aims of this paper were:

- To test the hypothesis that biochar incorporated into liquid dairy manure decreases manure GHG and NH_3 emissions.

- To test the hypothesis that biochar applied as a cap to liquid dairy manure decreases manure GHG and NH_3 emissions.

1.4.4 Chapter 5 (Paper 4): Impact of biochar on gaseous emissions and nutrient dynamics on soils in a California grassland dairy system

In order to assess the impact of biochar on a field scale, a one year field-based study was conducted. The main aims of this paper were:

- To test the hypothesis that biochar incorporated into sandy soils used to grow ryegrasses for silage will decrease nutrient leaching following manure application.

- To test the hypothesis that biochar incorporated into sandy soils used to grow ryegrasses for silage will decrease GHG and NH_3 emission following manure application.

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Chapter 2

Paper I: 'Establishing release dynamics for plant nutrients from biochar'

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Abstract

In order to assess the value of biochar to direct supply of crop nutrients we considered the release of phosphorus, magnesium and potassium from a hardwood biochar in a sequential leaching experiment with deionised water. Cumulative P release was proportionally large despite being quantitatively small, and the sixth extraction yielded 44-73% of the first, indicating that provision of soil P might be sustained for several seasons. Conversely, K release was quantitatively large but declined rapidly from first extraction to the last (6-18% of the first extraction). Only 6-27% of total Mg was recovered. These results indicate that these elements have contrasting associations with biochar that govern the trajectory and ultimate extent of their release. Fitting cumulative loss curves enabled these patterns to be quantitatively captured and compared and could provide a means to develop predictive capacity for the supply of nutrients from biochar to soil and plant.

2.1 Introduction

The pyrolysis of biomass produces energy as well as a solid product (biochar), which may have the potential to simultaneously enhance soil and sequester carbon in agricultural fields (Lehmann 2007), including those used to grow bioenergy crops. Energy crop production and biomass removal can be sustained as long as the necessary nutrients are provided to meet the demands of the crop (Helmisaari et al. 2011; Wu & Liu 2012); biochar could potentially be useful in the supply of nutrients to these crops. However, a comprehensive understanding of the function of biochar in soil is lacking. This limits its production and use since, at the likely cost of production (Shackley et al. 2011), a specific and predictable value to the end-user must be demonstrated. Given the return on inputs demanded by growers, the short-term contribution of biochar to soil nutrient supply must be understood. Nutrient elements in biochar emanate from the source biomass. However, feedstock concentration does not provide a reliable measure of biochar nutrient value in crop production. This appears to be for three reasons. Firstly, the proportions of each element mineralised (as ash), co-stabilised with C and volatilised (possibly recoverable in condensable products) are not equal, being affected by the chemical forms of the element in the feedstock as well as pyrolysis temperature and conditions. Nitrogen, for example, is volatilised in proportion to carbon and is also associated with the carbon in the retained fraction, sharing its recalcitrance (Knicker 2010), whereas phosphorus and potassium are largely conserved, being converted into inorganic forms (thermally mineralised) and retained into biochar in particulate form. The second factor relates to material loss of feedstock in pyrolysis. While oxygen and hydrogen are eliminated preferentially to carbon, carbon retention can range between 90% to as little as 10% if flash pyrolysis and gasification technologies are included, with a consequent ten-fold difference in concentration of conserved nutrient elements in biochar.

The physical micro-structure of biochar is a third consideration that could crucially affect the contribution of biochar to crop nutrient supply, controlling access to soluble (mineralised) elements by soil solution, micro-organisms and plant roots. Dominance of

physical controls would render rules and methods based on elemental ratio or chemical extraction used to assess biomass or soil organic matter inadequate. Although the contribution of biochar porosity and pore connectivity to this control of biochar nutrient supply is primarily a function of feedstock, it may be modified by the deposition of tars and formation of secondary char which is governed by the pyrolysis process and process parameters. Since the physical properties of biochar are modified after addition to soil, the propensity of biochar to weather, fragment and mineralise on a relevant timescale are likely to be relevant. To address the complex interactions between the first two factors and the third and create the necessary generalised understanding of biochar in nutrient supply, we propose a direct assessment that captures dominant physical controls (leaching) relative to biological or chemical processes (decomposition and desorption).

Prior attempts to quantify plant uptake and biochar release of nutrients in soil have produced inconsistent results (Chan et al. 2007; Steiner et al. 2007; Chan et al. 2008; Gaskin et al. 2010). We propose that existing understanding can predict the influence of soil pH and mineralogy on the fate of soluble nutrients released to soil and that, given the predominance of mineral forms in biochar and the evidence for a direct interface of roots and biochar (Prendergast-Miller et al. 2011), an appropriate assessment procedure for biochar will focus on the physical accessibility aspect. Repeated leaching approaches have been previously applied to the comparison of manures and composts (Sharpley & Moyer 2000), as well as to quantify the removal and recycling of inorganic plant nutrients from biochar (Wu et al. 2011). If patterns of nutrient release can be described in a way that creates a generalised understanding for different biochar feedstock and pyrolysis conditions, an expectation may be developed for the value of as yet unknown products, reserving agronomic trials for shortlisted products.

2.2 Materials and methods

In this study the magnitude and dynamics of short-term phosphorus (P), magnesium (Mg) and potassium (K) release was assessed with repeated cold water extraction using 10 g samples of hardwood biochar. Different levels of prior physical disruption were applied to establish the potential enhancement of release by post-application macro-fragmentation. To determine whether shaking during extraction caused abrasion or mechanical weathering (micro-fragmentation) the extractions were also made by simple soaking.

2.2.1 Biochar

The feedstock was hardwood *Acer pseudoplatanus* which was split into quarters and cut into segments less than 1 m in length. The feedstock was converted in a traditional charcoal ring kiln with a holding time of 24 h and a peak temperature of approximately 500°C (Dalkeith Charcoal, Edinburgh, UK). Proximate analysis was carried out in accordance with British Standard BS ISO17246:2010. Total elemental composition of both the feedstock sycamore and biochar was determined by aqua regia digestion followed by extraction with 2.5% acetic acid and ICP-OES analysis using a Perkin Elmer Optima 5300DV instrument (Perkin Elmer, Cambridge, UK) at an RF power of 1300 W and with plasma, auxiliary and nebuliser argon gas flows of 15, 0.2 and 0.75 L min^{-1} respectively, and a pump flow rate of 1.5 mL min^{-1}. Multi-element calibration standards (0.1, 1.0, and 10.0 mg L^{-1}, prepared from stock standards of 1000 mg L^{-1}) were used and the emission intensity measured at appropriate wavelengths. For all elements, analytical precision (RSD) was typically 1-5% for individual aliquots (n = 3). The mean value of the three aliquots was used for each sample value. Values from blank samples were subtracted from sample values.

2.2.2 Biochar nutrient extractions

The sample was divided into four particle size fractions by manual crushing and sieving: 0.15–0.60 mm, 0.60–1.18 mm, 1.18–4.00 mm and >4.00 mm. Biochar that is sold to

farmers may come in a range of sizes from a fine powder to large pieces, often within the same bag, thus encompassing each of the particle size ranges described herein. Published field experiments have used biochar that has been ground and sieved to particle sizes less than 2 mm (Liu et al., 2012; Zhang et al., 2012) to less than 10 mm (Major et al., 2012), though many field experiments have not described particle sizes (Castaldi et al., 2011; Scheer et al., 2011; Taghizadeh-Toosi et al., 2012). For each size in the present experiment, fraction 10 g sub-samples were weighed to 2 d.p. into 24 x 500 mL LDPE bottles. Initial moisture content of biochar was minimal and was not subtracted from initial weight calculations. Deionised water was added to each bottle in the amount that provided a precise 20:1 mass ratio. Two bottles containing only 200 mL deionised water were also prepared.

Three replicates for each biochar size fraction and one water-only control were shaken using an orbital shaker for 4 h at 160 min^{-1} and ambient temperature (20°C). Three replicates for each biochar size fraction and a water-only control were manually agitated and stood for 4 h at ambient temperature.

After the 4 h period the contents of all bottles were passed through paper filters (No. 1, Whatman, Maidstone, Kent, UK 11 µm retention) to recover biochar for repeat extraction, including secondary detached or mobilised fragments. This was done without dilution of the extract with additional water, but loss of material that was unable to be retrieved from the interior of the bottles was quantified prior to the next stage by re-weighing after drying overnight at 105°C and cooling in a desiccator. Filtrates were cleaned by passing through 0.45 µm membrane syringe-driven filters (Millex MCE sterile 33 mm diam., Millipore, Billerica, MA, USA) for analysis of dissolved P, K and Mg by ICP-OES as above. Mg, P, and K values from the control were subtracted from sample values. Cumulative values for each sample were determined by adding the results of all extractions for that sample.

The shaking or soaking and filtration / recovery sequence was repeated five times using the dried material recovered from the filters. The amount of biochar that was unable to

be retrieved from the filter paper was negligible. The volume of water added to the bottles in each cycle was adjusted to reflect the mass of remaining biochar material and maintain the 20:1 mass ratio.

To determine the extent to which shaking and soaking treatments had affected weathering and abrasion of biochar, dried samples at the end of the sixth cycle were sieved using the mesh sizes used at the beginning of the experiment and the proportion of particles that had moved from the original size category into smaller size fractions gravimetrically assessed.

2.2.3 Statistical analysis and curve fitting

The ICP results were used to generate release profiles and cumulative release curves for each element and biochar sample. One- or two-compartment kinetic models were fitted to the release profiles based on empirical determination and fitted using nonlinear least squares method and fit assessed using graphical analysis of residuals and a Shapiro-Wilk test for normality.

To assess the effects of particle size and extraction method on nutrient release rates, two-way analysis of variance (ANOVA) of cumulative results and release rate constants (from fitted curves) was performed with particle size, handling method and their interaction included as fixed effects. Where two compartments contributed to the overall release pattern, i.e. in two-component kinetic model fits, the dominant release rate in the initial part was used in analysis.

SigmaPlot 11.0 software was used for all analyses (Systat Systems Inc., 2008) and $P = 0.05$ selected in determining statistical significance.

2.3 Results

2.3.1 Biochar composition

Proximate analysis results for the char of volatiles, fixed carbon and ash were as follows: 18.5%, 77.0% and 4.4%, respectively. Carbon, hydrogen, oxygen, nitrogen and sulphur contents were 746, 28, 178, 4.0, and 0.2 mg kg^{-1}, respectively. Chlorine content was less than the detection limit (3 mg kg^{-1}). Phosphorus concentration in the biochar (119 mg kg^{-1}) was approximately four times greater than in the *Acer* spp. feedstock, reflecting the ratio of feedstock mass to biochar yield in biochar manufacture. In contrast, the Mg and K contents of 1889 and 3309 mg kg^{-1}, respectively, were only twice those of the feedstock.

2.3.2 Macro-fragmentation and loss

The range in mass loss resulting from six extractions and biochar recovery cycles was 11.4–20.6 % for the shaken samples and lower for the samples extracted by simple soaking, 5.1–15.3 %. The majority of this mass loss was due to biochar that was unable to be retrieved from the interior of the LDPE bottles or loss of sub-11µm particles that had passed through the filter paper. A negligible amount of biochar was unable to be retrieved from the filter paper after the biochar had been dried at 105°C.

2.3.3 Potassium release

Cumulative K release with repeated extraction ranged from major to complete (2400–4480 mg K kg^{-1}; Fig. 1a,b), more than half of which was recovered in the first cycle. The amount of K recovered from the *Acer* spp. biochar would equate to approx. 20–50 kg K ha^{-1} in topsoil assuming a biochar application rate of 20 t ha^{-1}. There was a marked effect of particle size, though only for the first extraction: smaller particles released K faster.

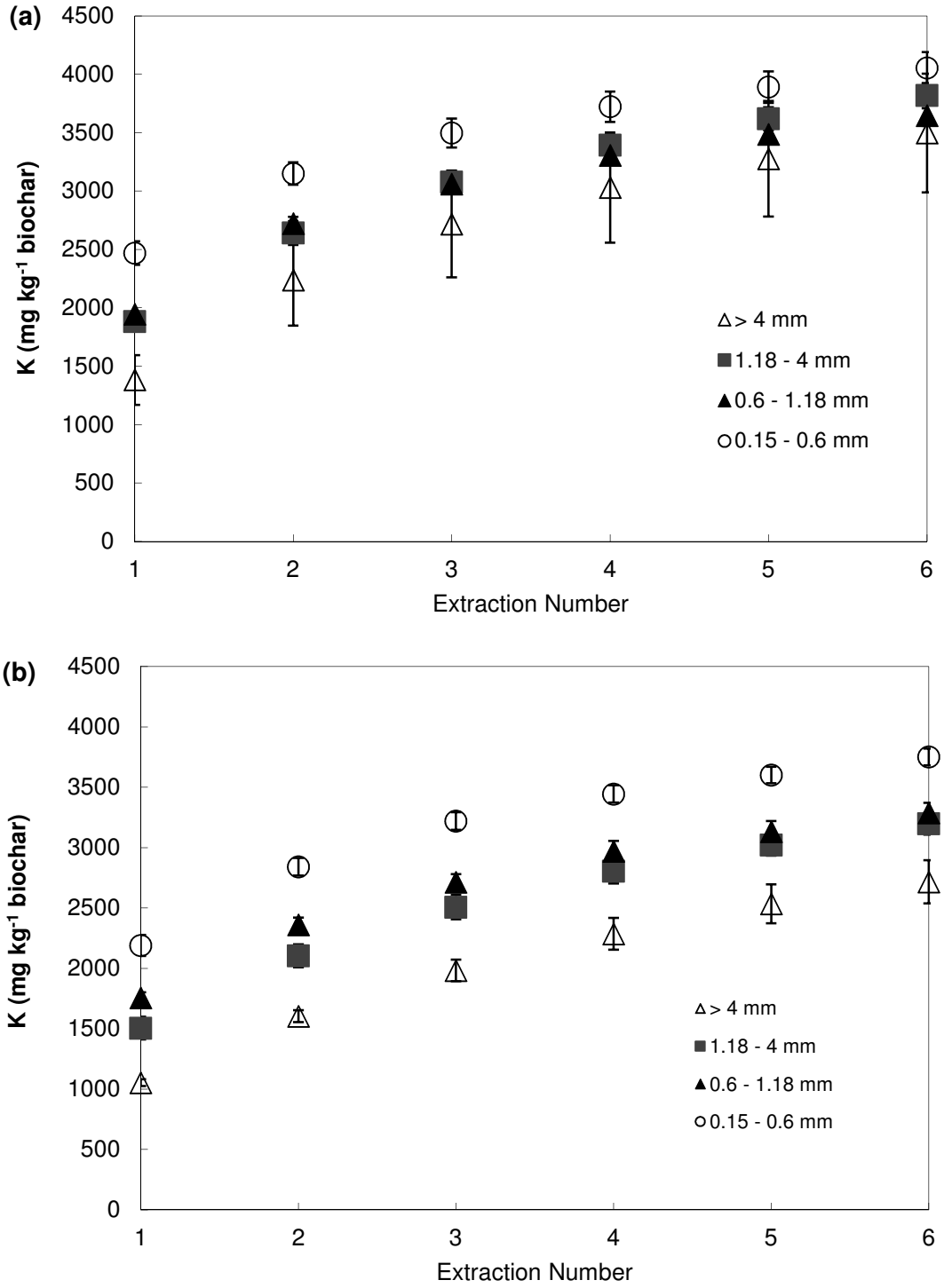

Figure 1: Cumulative release of potassium from (a) shaken biochar and (b) soaked biochar samples of four different particle size ranges, means and standard errors (n = 3)

2.3.4 Phosphorus release

Cumulative P release among the extractions ranged from partial to complete (30–130 mg P kg^{-1}; Fig. 2a,b). Although equating to only < 2.6 kg ha^{-1} (assuming 20 t ha^{-1}, application rate), the release of P was gradual and sustained: indeed, the amount did not change greatly between extraction cycles, suggesting potential for additional release from all samples with further extraction. The release of P was affected by particle size, being higher for smaller particles, that for the 0.15-0.60 mm particles being twice that for the > 4.00 mm fraction. Although shaking released more P than soaking, this was most apparent for the finest and largest fractions.

The trajectory of cumulative release between particle sizes suggested that P release might be sustained over time in the field, with certain fractions exceeding the nominal total content of biochar with repeated extraction, others reaching an asymptotic position below it.

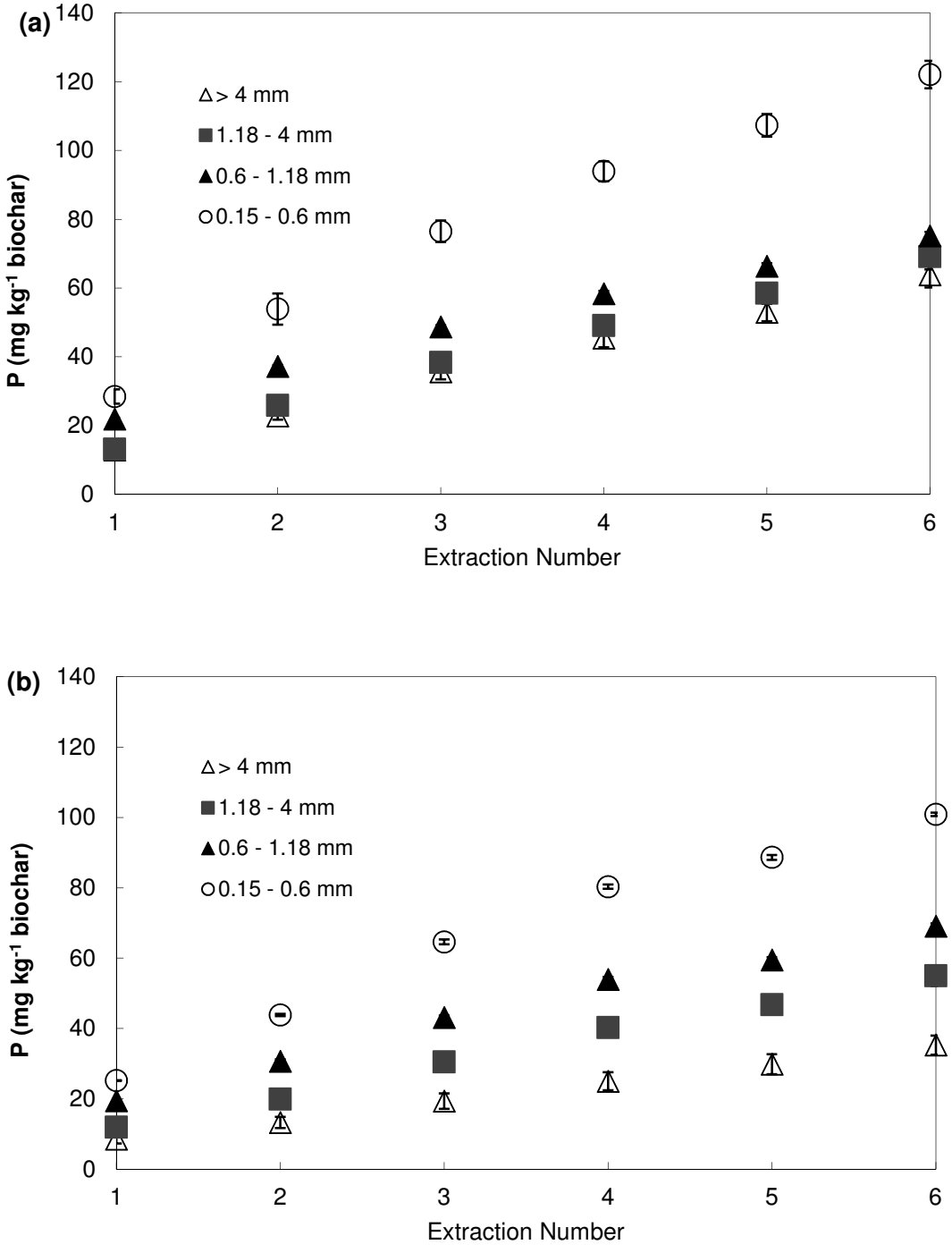

Figure 2: Cumulative release of phosphorus from (a) shaken biochar samples and (b) soaked biochar samples of four different particle size ranges, means and standard errors ($n = 3$)

2.3.5 Magnesium release

The relative range of Mg release was similar to that of P, the proportion higher and total amount lower (Fig. 3a,b). Cumulative Mg release was between 113-580 mg kg^{-1}, or at most one-third of total Mg. For the biochar used in this study, an application of 20 t ha^{-1} of sycamore biochar would result in a near-term release of 0.60-3.34 kg ha^{-1} of Mg to the soil.

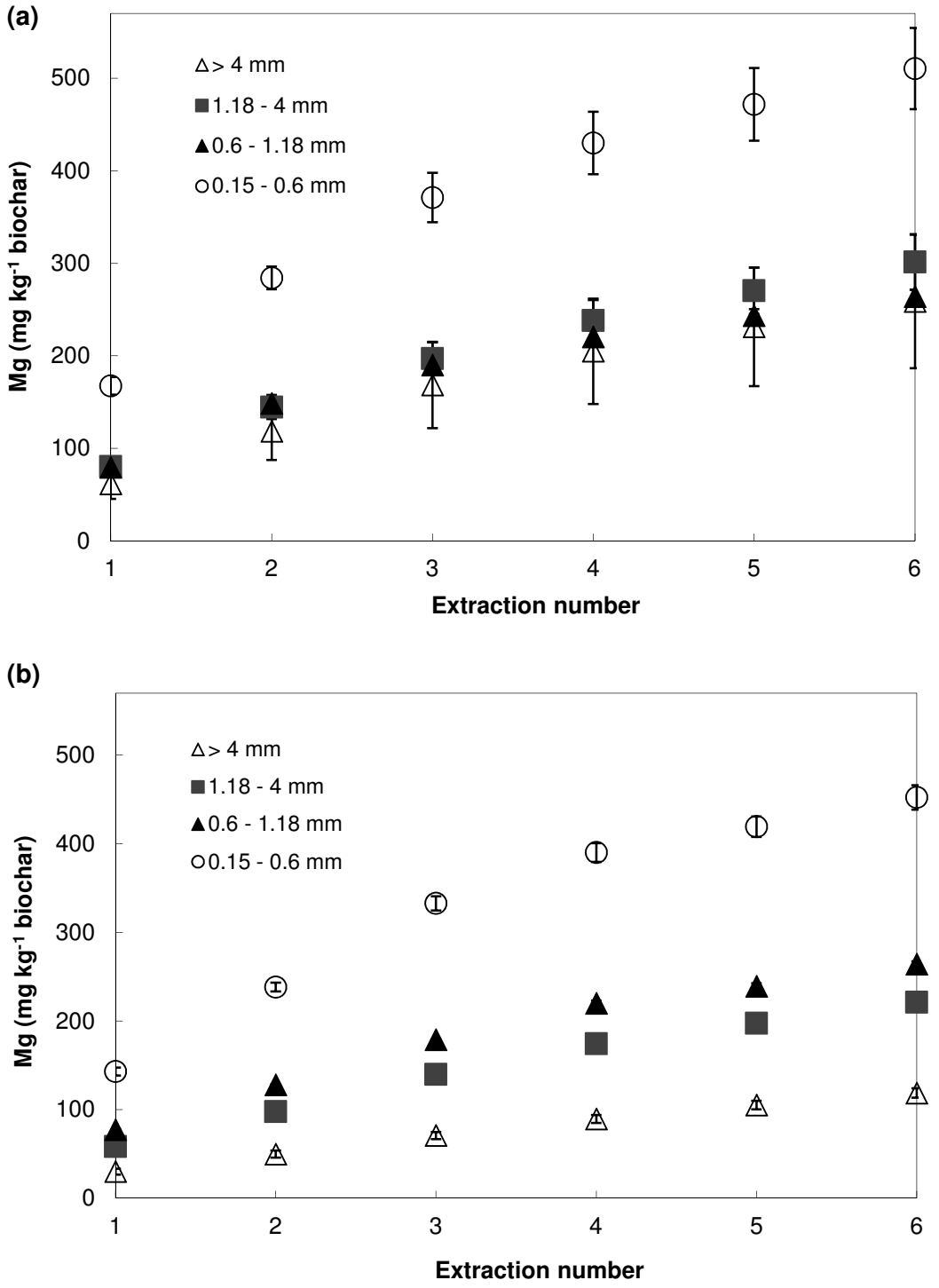

Figure 3: Cumulative release of magnesium from (a) shaken biochar samples and (b) soaked biochar samples of four different particle size ranges, means and standard errors ($n = 3$)

2.3.6 Statistical analysis and curve fitting

The constants indicative of rate of decrease in nutrients released with each additional extraction are shown in Table 1.

Analysis of variance applied to these fitted curve parameters indicated that particle size affected the release rate for K, P and Mg (smaller particle sizes released nutrients at a faster rate than larger particle sizes), and extraction method had significant effect on the ultimate release for K and Mg (shaking resulted in higher cumulative values than soaking) – but not P. Importantly, there was no significant interaction between the extraction method and particle size for these curve parameters.

Table 1

Release rate constants and r-squared values from the models fitted to release profiles of plant nutrients from sycamore biochar, means and standard errors ($n = 3$)

	Phosphorus		Potassium (dominant curve)		Magnesium	
	k	r^2	k_1	r^2	k	r^2
Shaken Samples						
> 4.0 mm	0.059 ± 0.029	0.05 - 0.53	0.678 ± 0.081	0.99 - 1.00	0.186 ± 0.014	0.90 - 0.95
1.18 - 4.00 mm	0.062 ± 0.006	0.27 - 0.72	1.674 ± 0.357	1.00	0.216 ± 0.020	0.92 - 0.98
0.6 - 1.18 mm	0.233 ± 0.005	0.90 - 0.93	1.111 ± 0.075	1.00	0.305 ± 0.011	0.96 - 0.97
0.15 - 0.6 mm	0.160 ± 0.040	0.78 - 0.91	1.963 ± 0.318	1.00	0.335 ± 0.028	0.97 - 0.98
Soaked Samples						
> 4.0 mm	0.079 ± 0.033	0.03 - 0.62	1.748 ± 0.159	1.00	0.145 ± 0.029	0.73 - 0.90
1.18 - 4.00 mm	0.077 ± 0.009	0.27 - 0.55	2.449 ± 0.349	1.00	0.183 ± 0.008	0.83 - 0.92
0.6 - 1.18 mm	0.188 ± 0.011	0.55 - 0.77	1.964 ± 0.188	1.00	0.247 ± 0.010	0.88 - 0.91
0.15 - 0.6 mm	0.174 ± 0.002	0.78 - 0.81	2.167 ± 0.289	1.00	0.306 ± 0.007	0.92 - 0.95

2.4 Discussion

The extraction process created fine particles and led to some losses of sub-11µm particles passing through the filter paper. In addition, some biochar was unable to be retrieved from the interior of the bottle following each extraction.

The high retention of P from the feedstock into biochar is consistent with other studies (Bridle & Pritchard 2004; Houssain et al. 2011; Wu et al. 2011). The lower proportional recovery of K and Mg suggests that either pyrolysis of these elements decreases extractability to a level that it is not extractable using methods applied to the analysis of soils, or that K and Mg were partially volatilised into gaseous (or condensable liquid) fractions. Higher retention of K and Mg in pyrolysed wood was demonstrated in Wu et al., indicating that K and Mg recovery are similar to that of P. It is possible that biochar directly influences the results by sorbing the extractants, as has been observed in microbial biomass studies (Durenkamp et al. 2010), whereas Wu et al. determined elemental composition of biochar by ashing and borate fusion. This could potentially explain why the total P and K amounts determined by ICP-OES analysis were exceeded by cumulative P and K results for many of the samples.

The recovery of all three elements (P, Mg, and K) was greater for smaller particle size fractions than larger particles. However, since there was no interaction with shaking versus soaking and only small proportions of material moved between size classes (based on direct assessment), this is likely to be due simply to the fact that smaller particles provide greater access to internal pores and the barriers of the physical structure of biochar, rather than as a consequence of direct abrasive removal of nutrient-containing ash.

The shaking of the biochar affected the release of Mg and K, but did not affect the initial release of P. Evidently, physical abrasion or weathering aids in the release of these elements. This may be due to the movement of the deionised water in the shaken samples

into smaller pore spaces coupled with differences in location of these elements within the biochar structure.

One or more of the elemental release profiles may have been affected by the loss of 0.45-11 µm particles that passed through the filter paper and were neither retained for future extractions nor collected as filtrate for analysis.

Based on mean cumulative amounts and the initial elemental characterisation, approx. 30-103% of the total P, 6-27% of the total Mg, and 82-122% of the total K was leached from the biochar in 6 extractions. These figures are consistent with Wu et al. (wood-based biochar prepared at 500°C) when calculated as a percentage of feedstock concentrations, assuming a 75% mass loss during pyrolysis. This indicates that the availability of Mg is low in pyrolysed material compared to that of P and K; the availability of Mg may be in a relatively insoluble form or hindered by the hydrophobicity of the biochar.

Despite the low P concentration in the biochar, the relatively large proportion of the total extractable P released suggests that P is conserved and rendered more water-available by pyrolysis relative to Mg. Although the amount of P released is relatively low, the ratio of the sixth extraction to the first extraction is high, indicating that P release may continue for several seasons.

The high rate constant of K and the relatively low ratio of release of the sixth extraction to that of the first extraction indicate that the water-available portion of K is released very quickly due to the high solubility of K-containing salts. It is unlikely that K from a biochar application to soil would be available beyond the first year following application. A high rate of K release and short-term availability in the soil are consistent with other published results (Gaskin et al. 2010; Silber et al. 2010; Yao et al., 2010). The high release of K from the smallest particle range in the first extraction also indicates that K is more easily leached from pores in small particles.

2.5 Conclusions

Although the P concentration in the biochar was low, the concentration of P in the feedstock and approximate ratio of feedstock mass to biochar yield indicates that most of the P is preserved in the pyrolysis process, whereas approximately half of the K and Mg is lost or unable to be retrieved through chemical extraction. Although cumulative P release was relatively small, the ratio of release from later extractions to release from earlier extractions was higher than the other elements, indicating that this release may be sustained for several seasons. Conversely, most of the available K was released at a very high rate, diminishing the supply quickly due to the high solubility of K-containing salts. Most of the Mg in the biochar was rendered unavailable by pyrolysis and a relatively small proportion of the total Mg was leached from the biochar in six extractions. These results indicate that these three elements have different solubility and adsorb differently to the biochar, perhaps at different locations within the biochar structure.

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Chapter 3

Paper 2: 'Biochar diminishes nitrous oxide and nitrate leaching from diverse nutrient sources'

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Abstract

Manure generated by large scale livestock operations poses ecological risks in the form of water pollution and greenhouse gas emission. In order to assess the impact of biochar on coarse-textured soils under contrasting nutrient management regimes, a 55-day incubation was conducted using unplanted soil columns amended with either manure, slurry or fertilizer (plus un-amended control), each with or without biochar applied at 2% soil mass (dry weight basis). Under repeated leaching, the cumulative N_2O emission from the columns was significantly affected by the presence of biochar ($p < 0.0001$), though these data were not normally distributed. Results indicated that the biochar amended soils emitted significantly less N_2O than their un-amended counterparts, with the exception of soils amended with manure. The presence of biochar increased the pH of column leachate by 0.08-1.70 and significantly decreased the cumulative amount of mineral N leached from the soil. The presence of biochar significantly increased the amount of PO_4^{3-} in soil leachate, but there was no significant difference between the means for any of the amendments used on their own, relative to their biochar-amended counter-parts. The data demonstrate that biochar could potentially aid in the mitigation of N_2O emissions from certain soils and in N loss in leachate from soil amended with slurry or manure produced by livestock systems.

3.1 Introduction

Manure and slurry generated by dairy cattle are useful soil amendments for provision of N and P to crops, but pose numerous environmental risks including water pollution and release of greenhouse gas emissions (FAO, 2006). Nitrous oxide (N_2O) rates of emission from organic soil amendments such as manure and slurry can exceed those of mineral fertilizers (Senbayram et al., 2009). Globally, approximately 2% of the manure nitrogen that is added to soil is emitted as N_2O. As such, manure N has been an important contributor to the global increase of atmospheric N_2O (Davidson, 2009). N_2O has an atmospheric lifetime of 114 years, a Global Warming Potential (GWP) of 298 over a 100-year time horizon (Solomon et al., 2007), and is the single most important contributor to stratospheric ozone depletion (Ravishankara et al., 2009).

Nutrient leaching from manure that has been applied to soil is also an important source of water pollution (Stout et al., 2000; Buda and Klelnman, 2009). High levels of nitrogen may damage ecological systems (Vitousek, 1997) and contaminate groundwater (Spalding and Exner, 1993). Freshwater systems are also highly sensitive to pollution by phosphorus (P) runoff (Carpenter and Bennett, 2011). Indeed, management of P loading to surface waters is crucial to the prevention of eutrophication (Carpenter, 2008; Schindler et al., 2008).

Biochar is a carbon-rich solid product of biomass pyrolysis that is currently being investigated as a potential soil amendment and carbon sequestration technology (Lehmann, 2007). Biochar made from a range of different feedstocks and peak process temperatures has shown the potential to suppress N_2O emissions from several soil types (loam, clay loam, loamy sand, sandy loam, and silty loam), including soils that have not been amended with sources of N, or those that have been amended with commercial fertilizer (Case et al., 2012; Liu et al., 2012; Zhang et al., 2012), bovine urine (Taghizadeh-Toosi et al., 2011) or anaerobically digested cattle slurry (Bruun et al., 2011). Rogovska et al. (2011), for example, introduced dried swine manure (with and

without a mixed hardwood biochar) to fine loamy soil columns at day 79 of a 500 day incubation experiment and measured N_2O emissions on day 414. Their results indicated that N_2O emissions were suppressed by the presence of biochar at that point in the experiment. Other studies saw no significant impact of pine biochar on N_2O emissions from silt loam soil (Clough et al., 2010), or a reduction of N_2O flux from an Alfisol and Vertisol amended with wood-based and poultry litter biochar that was not immediate but, rather, was observed after several months and two wet-dry cycles (Singh et al., 2010).

Biochar amendment may reduce total nitrogen loss through leaching (Lehmann et al., 2003) and may reduce loss of ammonium through leaching (Ding et al., 2010; Singh et al., 2010). The potential to decrease nitrate loss from soils amended with biosolids (Knowles et al., 2011), to increase N use efficiency (Chan et al., 2007), and to reduce P loss through leaching has also been reported (Novak et al., 2009; Yao et al., 2011). The companion study to Rogovska et al. (2011), Laird et al. (2010), indicated that biochar decreased N and P loss in leachate from swine manure amended columns, even though the biochar itself added substantial amounts of these nutrients to the leachate from control columns with no added manure.

Most biochar research has focused on biochar used in conjunction with commercial fertilizer application to soils, rather than with manure or slurry. Some studies have included biochar made from pyrolyzed manure (Singh et al., 2010; van Zwieten et al., 2010; Uzoma et al., 2011; Rajkovich et al., 2012); have compared the use of biochar as an amendment with the use of manure or composted manure (Tanaka et al., 2006; Kimetu et al., 2008; Martins et al., 2010); or have examined the impact of biochar on the composting of manure (Chen et al., 2010; Steiner et al., 2010; Jindo et al., 2012). The available literature which addresses the simultaneous application of manure and biochar within the same soil treatment is limited and has not included repeated gas sampling events in conjunction with repeated leaching events. To date, there has been no comparison of the effect of biochar on farmyard manure with the effect of biochar on fresh (undigested) cattle manure slurry, an equally important organic fertilizer. As co-

application with other amendments may better accommodate the distribution of biochar on the field scale (and prevent loss of fine particles), the N_2O emissions from and nutrient dynamics in soils that have been amended with a mixture of biochar with manure, slurry, or commercial fertilizer are here investigated.

The purpose of this study is to: (i) examine the impact of fresh biochar on N_2O emissions from soil alone or amended with mineral fertilizer, manure, or slurry and (ii) examine the impact of biochar on nutrient retention from soil amended with mineral fertilizer, manure, or slurry.

3.2 Materials and methods

The present N_2O and repeated leaching study was conducted using soil leaching columns and the greenhouse facilities at University of Edinburgh, Scotland, UK. Average daily minimum in the greenhouse throughout the course of the 55 day experiment was 23.0ºC with an average daily maximum of 32.0º C. Sandy loam soil (Cambic Arenosol) was obtained in April 2010 from the Rothamsted Research Woburn Experimental Station, Woburn, Bedfordshire, UK. The soil was sampled along a cross-section of the field. Soils in this field have been classified as the Cottenham series, the Stackyard series, the Flitwick series, and the Husborne series. The soils are further described in Catt et al. (1979). This soil has not been cultivated for approximately 60 years resulting in low total organic carbon (TOC) (10.2 g kg^{-1}), total N (380 mg kg^{-1}), and PO_4^{3-}-P content (5.8 mg kg^{-1}). After collection, the fresh soil was passed through a 6 mm mesh sieve.

Biochar was obtained as charcoal from Dalkeith Charcoal Ltd., Edinburgh. This particular sample was produced from sycamore (*Acer pseudoplatanus*), heated to 500ºC for 24 hours in a traditional charcoal kiln. The biochar was ground using a pestle and mortar and sieved to a 1.18-4.00 mm size range to reflect the particle size that would be likely to be applied to a field by farm equipment. The biochar contained a total amount of 746 g C kg^{-1}, 4.7 g N kg^{-1}, and a PO_4^{3-}-P content of 118 mg kg^{-1}.

Dairy cattle manure and slurry were obtained from the Crichton Royal Farm of the Scottish Agricultural College, Dumfries, Scotland, UK. In order to achieve a greater homogeneity, a few mL of deionized water was added to the manure, and the mixture was thoroughly blended with a blade grinder. Blended manure contained 21% dry matter with TOC content of 280 g kg^{-1}, 12.8 g N kg^{-1}, and 1170 mg kg^{-1} PO_4^{3-}-P. Slurry contained 11% dry matter with TOC content of 460 g kg^{-1}, 36.5 g N kg^{-1}, and 2690 mg kg^{-1} PO_4^{3-}-P (based on dry weight). The dry matter content of slurry from the Crichton Royal Farm is higher than typical of British dairy farms due to a concerted effort to decrease water

use. Soil, biochar, manure, and slurry were sub-sampled for analysis prior to the start of the experiment in order to determine the above properties.

Eight treatments (including the control) were applied to 2 kg of soil (dry weight equivalent) with four replicates, randomized spatially (no blocking) within the allocated greenhouse space. Treatment codes were assigned as follows: soil-only (control), soil plus biochar (B), soil plus fertilizer (F), soil plus fertilizer and biochar (FB), soil plus manure (M), soil plus manure and biochar (MB), soil plus slurry (S), and soil plus slurry and biochar (SB). Soil for each replicate column was prepared separately, being weighed out and then mixed with the relevant amendment(s) where applicable, using a trowel. Biochar was mixed into the soil at a rate of 2% based on dry weight and for MB and SB treatments, mixed with the manure or slurry prior to addition. Deionized water was added to biochar and manure in order to match their gravimetric water holding capacity prior to application to the soil, to avoid the amendments drawing water from the bulk soil after addition. For biochar, water holding capacity was taken to be 100% (dry weight basis), recognizing that only part of the capacity of biochar to hold water outside the soil is available after addition. Manure, slurry and commercial fertilizer (as NH_4NO_3) were mixed into the soil at rates based on equal total N per column (66.0 mg N) and referred to generically as the soil "N source". In addition, all F and FB columns received commercial fertilizer at 108.8 mg of $Ca(H_2PO_4)_2$ (the equivalent of 52 kg P ha^{-1}) and 126.4 mg of KCl (the equivalent of 120 kg K ha^{-1}). These additions collectively supplied N, P, and K at the area-based rates of 120, 52, and 120 kg ha^{-1} (respectively) as per standard fertilizer recommendations for sandy soil sown to winter barley (DEFRA, 2010). The area-based rates were scaled to account for the calculated soil depth in the column relative to plough depth in the field (approx. 23 cm). The amounts of biochar C, total N, mineral N, and PO_4^{3-}-P are presented as Table 1.

Table 1: Nutrient application rates of amendments used in the study (g per column)

Treatment	Total Organic Carbon	Total N	Extractable N	Extractable P
Control	0	0	0	0
Biochar	29.84	0.188	0.0001	0.0047
Fertilizer	0	0.066	0.066	0.028
Fertilizer + biochar	29.84	0.254	0.066	0.033
Manure	0.0014	0.066	0.012	0.006
Manure + biochar	29.84	0.254	0.012	0.011
Slurry	0.0008	0.066	0.033	0.0049
Slurry + biochar	29.84	0.254	0.033	0.0096

Soil and amendments were transferred to 35-cm tall columns, made from 11 cm diam. polyvinyl chloride piping. The columns were packed evenly by hand to a depth of approx. 16 cm (no biochar amendment) or 17 cm (for columns that contained biochar). The base of each column was lined with two layers of nylon mesh and one layer of cotton muslin, clamped to the column by a pipe connector that also served to elevate the soil and cloth liner from the table surface approx. 5 cm and enabling leachate to be captured in a collection dish.

Deionized water was added slowly to each soil column in order to bring the soil to 100% gravimetric water holding capacity (WHC). All of the columns started leaching prior to attaining the calculated 100% WHC, so the weight of the column was recorded at the point leaching began and no further water was added. These recorded weights were considered to represent 100% WHC for the columns for the remainder of the experiment.

In order to maintain constant moisture of the soil between leaching events, columns were weighed every 2-3 days and adjusted to 100% WHC using deionized water. Pierced

Parafilm (Pechiney Plastic Packaging Company, Chicago, IL, USA) was affixed to the tops of each column using a rubber ring to limit soil drying while permitting gas diffusion.

Headspace gas samples were taken 21 times (2–3 day intervals) over a 55-day experimental period. At the beginning of each gas sampling event, 50 mL of water were added to the leachate collection dish to create an air-tight seal below the column. Headspace chambers comprising 25 cm sections of additional piping with airtight lid, sampling port and 3-way stopcock were connected to the top of each column with a rubber ring and the time was recorded. After 30 min closure a headspace gas sample was taken for each column using a vacuum hand pump and a 20-22 mL glass vial sealed with a crimp cap and chloro butyl rubber septum (Chromacol, Welwyn Garden City, UK). The vials were filled from the headspace and evacuated to 70kPa twice prior to keeping the third sample for analysis. An ambient air sample was taken within the greenhouse at each sampling event and an analysis of linearity of N_2O emissions conducted weekly. Gas samples were analyzed for N_2O using an Agilent 6890 Series Gas Chromatograph (Santa Clara, CA, USA). N_2O was calculated from ion chromatograph data by linear regression using standards of 0.3, 0.5, 1.1, 2.7 and 10.2 ppm. Linearity analysis was conducted by sampling from one column once every 5 min for the period of 1 h. The results were plotted and a linear regression conducted for analysis.

The columns were leached once per week. During each leaching event, each column received 100 mL of deionized water, added slowly and evenly over the surface of the soil using a 10 mL syringe. The leachate was allowed to drain through the column for a minimum of 5 h. An additional 50 mL of deionized water was added to each collection dish at the time of gas sampling (approx. 3-4 h after the start of the leaching event) in order to ensure that there was enough water for an airtight seal at the bottom of each column. One collection dish was left throughout the duration of the sampling event on the same table, containing 150 mL of deionized water as a blank. At the end of each sampling event, leachate volumes were recorded and a representative aliquot of 25 mL was stored below 4° C until the time of analysis (generally less than 1 week).

Soil samples were taken prior to packing each column (after amendment application) on day 0, and after dismantling each column on day 55. The soil was thoroughly mixed before the sample was taken.

3.2.1 Chemical analysis

Initial characterization of soil, biochar, manure and slurry, as well as day 0 and day 55 soil samples were analyzed for gravimetric moisture content, water holding capacity, and pH. The method for pH measurement of soil, manure, and slurry was based on BS 7555-3.2:1995. A 1:5 ratio mixture with deionized water (resistivity of 18.2 MΩ cm, Direct Q3 Millipore Water Purifier, Millipore, USA) was shaken on a wrist action shaker (SF1, Stuart Scientific, UK) for 10 min at 700 osc min^{-1} and left to settle for 2 h prior to analysis. The method of pH characterization of biochar was based on Shinogi and Kanry (2003). A 1:10 ratio mixture with deionized water was boiled for 15 min and allowed to cool to room temperature prior to analysis. pH of all materials was assessed using a PHM93 reference pH meter (Hach Lange, Manchester, UK) with sampling tray (SAC 80, Radiometer Analytical, Copenhagen). The pH electrode (Russell Mainstream Supply, Ladybank, UK) was calibrated against a series of pH buffers (pH 4, 7, and 10, Thermo Fisher Scientific, UK). TOC of soil, manure, and slurry was estimated by loss on ignition (Nelson and Sommers, 1996). Proximate analysis of this biochar was carried out in accordance with British Standard BS ISO172462010. Total N was determined by Kjeldahl digestion (Schumacher et al., 1997), extractable phosphate (PO_4^{3-}-P) was determined using 2.5% acetic acid extraction, and NH_4^+-N by extraction with KCl 1M in a 10:1 ratio. Total N, PO_4^{3-}-P, and NH_4^+-N concentrations were colorimetrically analyzed in their respective extracts using a KONE Supra chemical analyzer (Helsinki, Finland) using blanks, standards of 1, 5, 10, an 25 mg L^{-1} PO_4^{3-}P, and either 0.1, 0.3 and 0.6 mg L^{-1} or 1.2, 2.4, 4.6, 10.0, and 20.0 mg L^{-1} NH_4^+-N (depending on sample concentrations). NO_3^--N was determined using deionized water extraction, and analyzed using a DX-500 ion chromatograph (DIONEX, Sunnyvale, CA, USA) using blanks and standards of 1, 5, 10, and 25 mg L^{-1}. Cation exchange capacity was determined in accordance with British

Standard BS 7755-3.3:1995 using three extractions of 0.1 M $BaCl_2$, and one extraction each of 0.0025 M $BaCl_2$ and 0.02 M $MgSO_4$. The products were analyzed using ICP-OES (Perkin Elmer 5300 ICP-OES, USA) and the combined values of exchangeable K^+, Ca^{2+}, Mg^{2+}, and Na^+ were used to estimate total cation exchange capacity (CEC). Blanks and media-matched standards of 0.1, 1.0, and 10.0 mg L^{-1} were used in this analysis.

Leachate samples were analyzed for PO_4^{3-}-P and NO_3^--N using a DX-500 ion chromatograph (DIONEX, Sunnyvale, CA, USA). Samples were diluted, if required, to a concentration between 5 and 20 mg L^{-1} to allow a measurement in the calibration range. They were passed through an IonPac AS4A-5C analytical column for the separation of anions. Detection was done via an ED40 electrochemical detector. Leachate samples were analyzed colorimetrically for dissolved NH_4^+-N using the KONE Supra chemical analyzer (Helsinki, Finland). pH of leachate samples were measured without additional treatment using the pH equipment described above.

3.2.2 Calculation of cumulative results and statistical analysis

Cumulative N_2O emissions were estimated using the linear trapezoidal method. Cumulative leachate results were calculated by adding the results (minus a blank) for each leaching event. Average pH results for leachate and soil samples were calculated by converting pH results to $[H^+]$, calculating the arithmetic mean, and converting the mean back to pH. The relative gain or loss of soil TOC, total and mineral N and P, Ca, Mg, K, and CEC over time was calculated by using the ratio of the day 0 value to the day 55 value of the soil and reported as 'soil change ratios'. Mineral N results for soil and leachate samples were calculated by combining NO_3^--N and NH_4^+-N results. Parameters were summarized using arithmetic means and standard errors (SE).

The effects of nitrogen source and biochar on cumulative N_2O, cumulative leachate, pH, and soil change ratios were assessed using two-way analysis of variance (ANOVA). All parameters were log-transformed (natural log) in order to meet the assumption of normality prior to statistical analysis. All resulting data were normally distributed unless

otherwise noted below. Nitrogen source, biochar addition, and their interaction were included as fixed effects. Post-hoc comparisons were performed using Tukey's correction for multiple testing. Model fit was assessed using graphical analysis of residuals, a Shapiro-Wilk test for normality, and the O'Brien test for equal variance. The 0.05 confidence interval was used to determine statistical significance. All statistical analyses were conducted using JMP 9.0.0 (SAS Institute, Cary, NC, USA).

3.3 Results

3.3.1 Impact of biochar amendment on N_2O fluxes

N_2O emissions typically peaked approx. 2-4 days following each leaching event and decreased during each leaching event (Figure 1). Mean treatment cumulative emissions ranged from approximately 0.1 to 0.9 mg N_2O-N kg^{-1} soil (Figure 2). N_2O emissions were significantly affected by N source ($p < 0.0001$) and presence of biochar ($p < 0.0001$), but there was no interaction effect ($p = 0.211$). Although the N_2O data passed the equal variance test, they failed the normality test ($p = 0.0027$). Tukey test results indicated that only the emissions from the B treatment were significantly less than their non-amended counterparts (the control). An analysis of the residuals indicated that there was one outlier in the dataset, namely one replicate column in the MB treatment that displayed much lower N_2O flux (0.02 mg N_2O-N kg^{-1} soil) than the other MB samples (0.09-0.19 mg N_2O-N kg^{-1} soil) or even the B samples which had included no additional N source (0.04-0.08 mg N_2O-N kg^{-1} soil).

Performing the statistical analysis after removal of the outlier improved the graphical analysis of the residuals as well as the results of the normality test ($p = 0.6044$). These results indicated identical ANOVA results for the effects of N source and presence of biochar as the earlier test, but indicated an interaction effect ($p = 0.0014$). Tukey test results indicated that N_2O emissions from B samples were significantly less than those from control samples, and that emissions from FB and SB were significantly less than those of the F and S (respectively).

Linearity checks indicated that the concentration increases of N_2O released were linear during the incubation period.

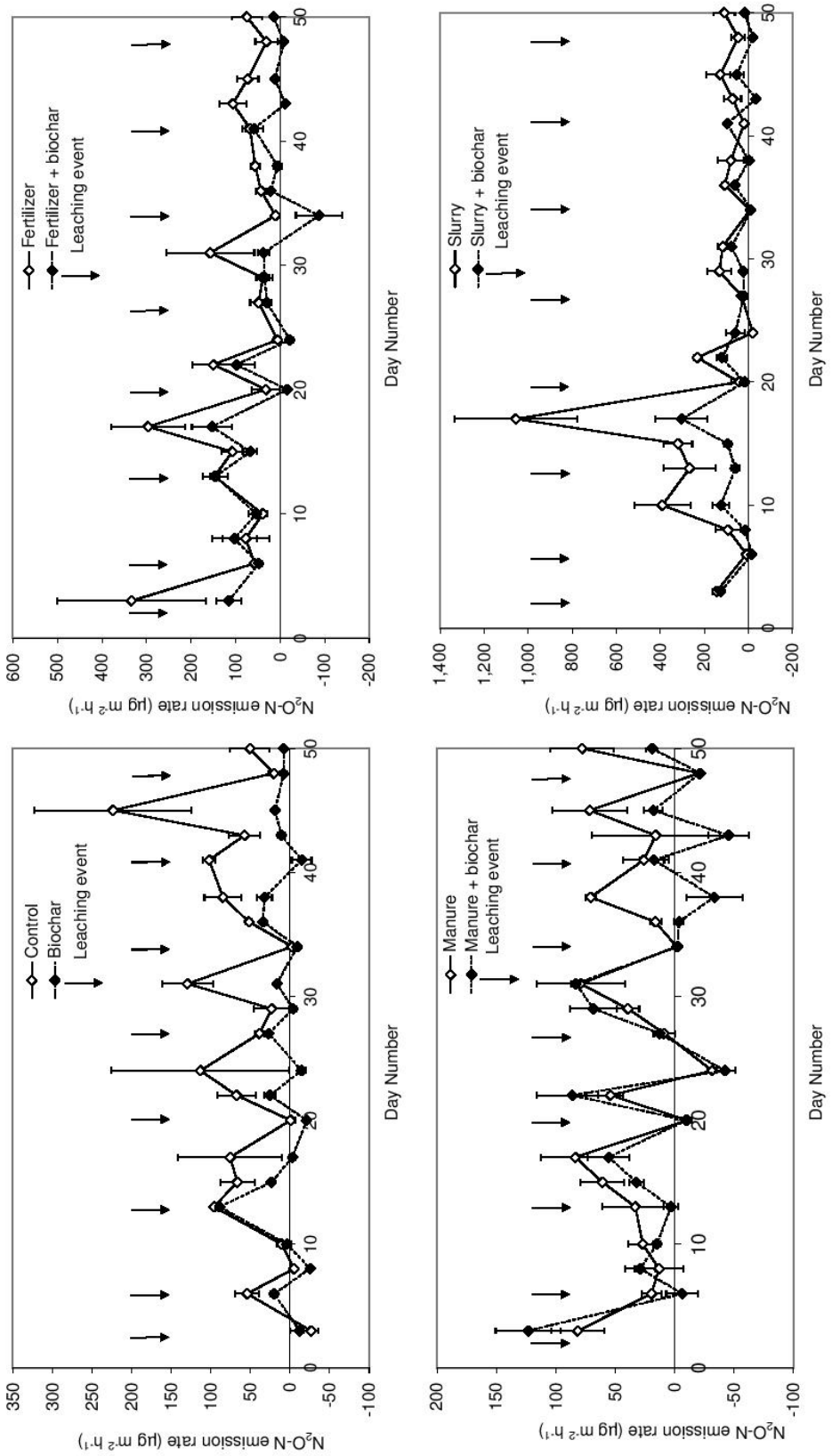
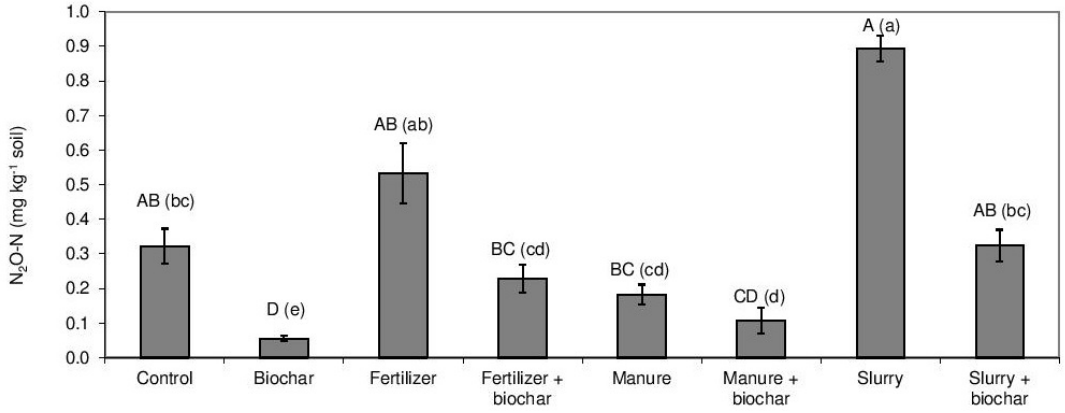


Figure 1: Effect of biochar on N_2O emissions from soil amended with fertilizer, manure, or slurry (n = 4)

Figure 2: Cumulative N_2O-N release in the greenhouse during 55 days. Results are means and SE (n = 4). Different letters indicate Significant differences (p <0.05) between the cumulative results of treaments (Tukey's correction for multiple testing). Letters in parentheses indicate Tukey test results after removal of one outlier



3.3.2 Leachate pH and nutrients

Biochar increased average leachate pH for all treatments at each time point by 0.08–1.70 units (Figure 3) and Tukey test results indicate that the pH of leachate samples from all biochar-amended treatments were higher than their non-amended counterparts for all leaching events, except at day 13 and day 20. Presence of biochar had a significant effect on the pH of the leachate for all leaching events (as did N source) and a significant interaction effect between biochar and N source was evident during 5 of the 8 leaching events. Data from day 13 and day 20 failed the normality test and data from day 20 failed the equal variance test.

Presence of biochar significantly increased (p = 0.0325) cumulative PO_4^{3-}-P in leachate and significantly decreased (p < 0.0001) cumulative mineral N in leachate (Table 2; Figures 4, 5, 6). There was a statistically significant interaction between presence of biochar and N source for both PO_4^{3-}-P and mineral N results (p = 0.0413 and p < 0.0001 respectively). Cumulative mineral N in leachate (for which treatment mean values ranged from 1.2 - 54.5 kg ha^{-1}) were significantly lower from biochar amended columns than from their un-amended counter-parts for all sources of N, and average cumulative results were lower for both NO_3^- and NH_4^+ from biochar amended soils (data not shown). Biochar decreased mineral N in leachate from fertilizer-amended columns by approx.

44.6%, from slurry-amended columns by approx. 48.3%, and from manure-amended columns by approx. 66.7%. Proportion of total N (added as amendments) leached over the duration of the experiment was lowest from B and MB columns (both at 0.6%) and highest from S and F columns (78.5% and 31.6%, respectively). Average cumulative P in leachate (which ranged from 0.10 - 0.32 kg ha^{-1}), were higher from B, FB, and SB columns than from the control, F, and S columns (respectively), but average PO_4^{3-}-P from MB columns was lower than average PO_4^{3-}-P from M columns. However, these differences between mean values for PO_4^{3-}-P were not significant. Proportion of PO_4^{3-}-P (added as amendments) that was leached over the duration of the experiment was lowest from F and FB columns (1.2% and 2.8%, respectively) and highest from M and B columns (13.5% and 15.8%, respectively).

Maximum rate of mineral N release from S and SB columns occurred during the third and fourth leaching events. Maximum rate of mineral N release for each of the other treatments occurred during the first leaching event.

Figure 3: pH of leachate from a sandy soil amended with inorganic fertilizers, manure, slurry and/or biochar, determined with repeated leaching events (each data point represents one leaching event) from soil columns; means and standard errors (n = 4)

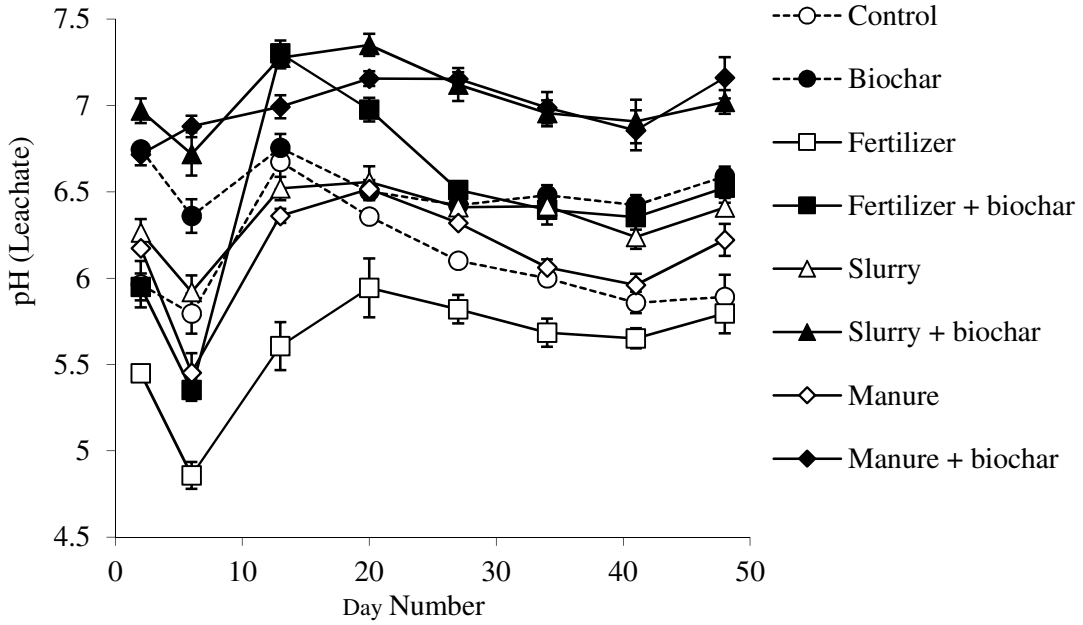


Table 2: Treatment effects on cumulative mineral N and PO_4^{3-}-P leaching from a sandy soil amended with inorganic fertilizers, manure, slurry and/or biochar, determined with repeated leaching events from soil columns in the greenhouse during 55 days; means and standard errors (n = 4)

	Mineral N (kg ha^{-1})	**PO_4^{3-}-P (kg ha^{-1})**
Control	5.6 ± 0.5d†	0.17 ± 0.05ab
Biochar	1.2 ± 0.1e	0.26 ± 0.04ab
Fertilizer	54.5 ± 1.7a	0.12 ± 0.03ab
Fertilizer + biochar	30.2 ± 3.1b	0.32 ± 0.08a
Manure	4.9 ± 0.6d	0.28 ± 0.02a
Manure + biochar	1.7 ± 0.1e	0.17 ± 0.02ab
Slurry	22.0 ± 0.9b	0.10 ± 0.04b
Slurry + biochar	11.4 ± 1.1c	0.15 ± 0.03ab

† Different letters indicate significant differences (p < 0.05) between the cumulative results of treatments (Tukey's correction for multiple testing, n = 4)

Figure 4: PO_4^{3-}-P concentrations of leachate from a sandy soil amended with inorganic fertilizers, manure, slurry and/or biochar, determined with repeated leaching events (each data point represents one leaching event); means and standard errors (n = 4)

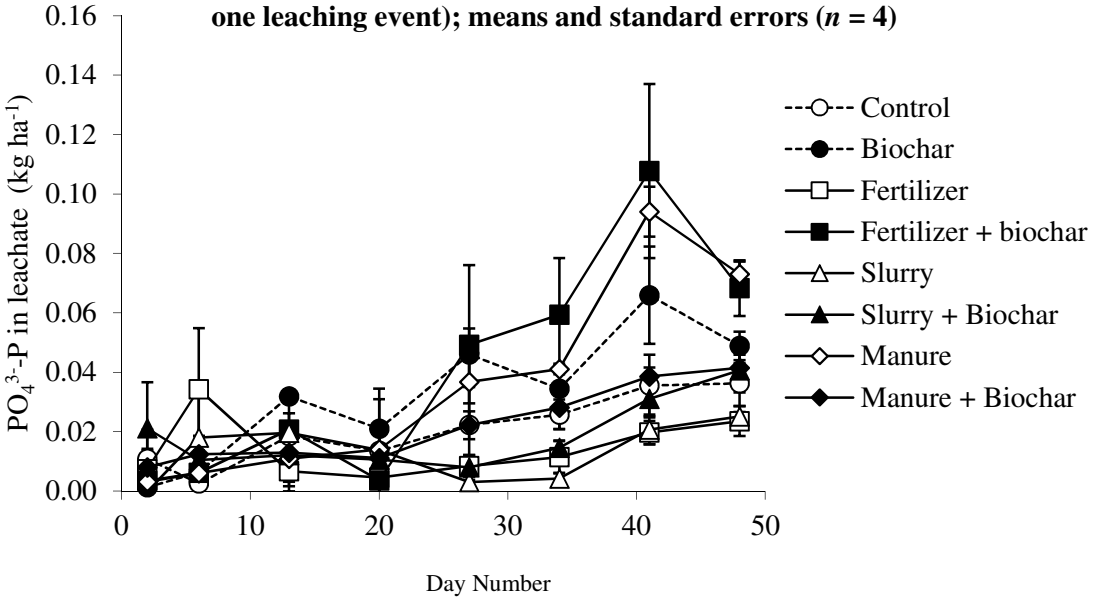


Figure 5: NH_4^+-N concentrations of leachate from a sandy soil amended with inorganic fertilizers, manure, slurry and/or biochar, determined with repeated leaching events (each data point represents one leaching event); means and standard errors (n = 4)

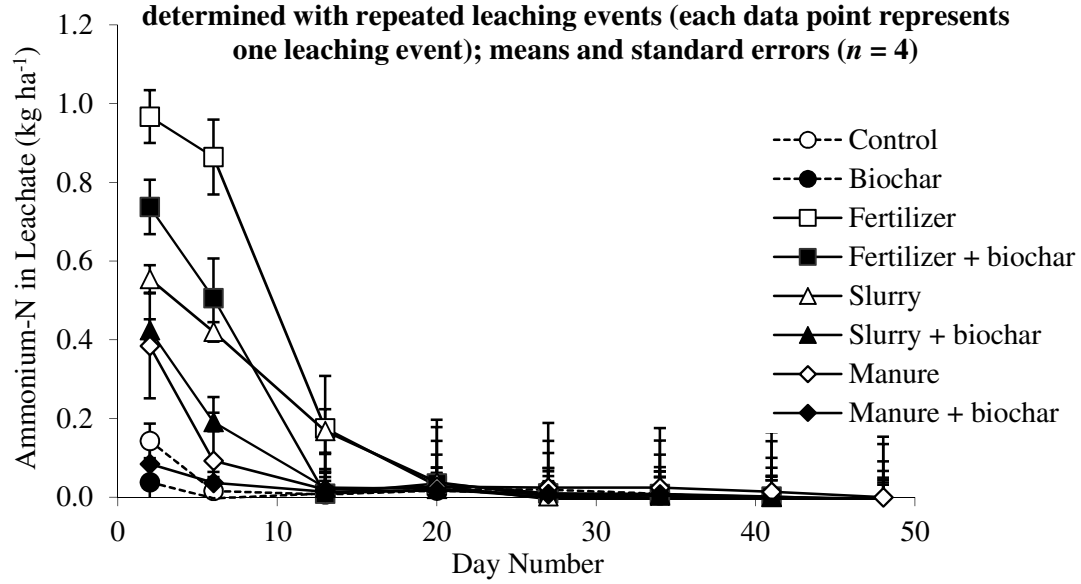
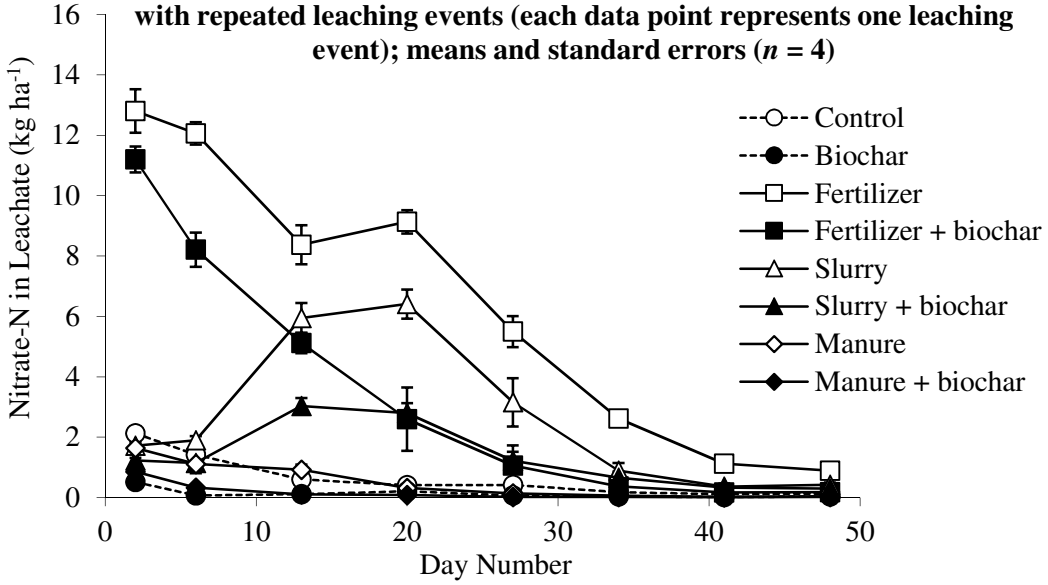


Figure 6: NO_3^--N concentrations of leachate from a sandy soil amended with inorganic fertilizers, manure, slurry and/or biochar, determined with repeated leaching events (each data point represents one leaching event); means and standard errors (n = 4)



3.3.3 Soil pH, carbon, and nutrients

In general, the pH of soils at day 55 was higher than at day 0, with the exception of the control, M and MB columns (Table 3). Biochar and N source significantly affected the pH of the soil for both sampling events, but the interaction effect that was evident on day 0 was not evident on day 55. The MB treatment had a significantly higher pH than the M treatment on both day 0 and day 55, and the SB treatment had a significantly higher pH than the S treatment on day 55. The pH data for day 0 failed the normality test (p = 0.0016).

Mean TOC contents for biochar-amended columns were higher than for their un-amended counterparts due to the high C content of the biochar, but the difference between day 0 and day 55 was less than the standard error for all treatments, with the exception of the MB columns. The soil change ratios indicated a slight increase in the mean TOC values of the control, B, F, FB, and SB columns and a slight decrease in the mean TOC values of the M, MB, and S columns (Table 3). Although biochar did not have a significant effect on the TOC change ratio, there may have been a weak interaction (p = 0.0757).

Table 3. Treatment effects on soil nutrient change ratios from a sandy soil amended with inorganic fertilizers, manure, slurry and/or biochar, determined with repeated leaching events from soil columns in the greenhouse during 55 days; means and standard errors (n = 4).

		Treatment							
Variable	**Day**	**Control**	**Biochar**	**Fertilizer**	**Fertilizer + biochar**	**Manure**	**Manure + biochar**	**Slurry**	**Slurry + biochar**
pH	0	6.7 ± 0.2ab†	6.3 ± 0.1ab	5.2 ± 0.0e	5.6 ± 0.0de	6.2 ± 0.2bc	6.8 ± 0.0a	5.6 ± 0.0de	5.9 ± 0.1cd
	55	6.5 ± 0.2ab	6.9 ± 0.1a	6.3 ± 0.2bcd	6.4 ± 0.1bc	6.0 ± 0.1cd	6.5 ± 0.0ab	5.9 ± 0.0d	6.4 ± 0.1bc
Total Organic Carbon (g kg^{-1})	0	10.2 ± 0.1	19.0 ± 1.2	10.0 ± 0.7	18.7 ± 1.2	10.1 ± 0.7	27.0 ± 1.9	10.6 ± 0.8	18.2 ± 2.8
	55	10.6 ± 0.6	21.9 ± 0.8	11.8 ± 0.8	21.9 ± 1.4	8.4 ± 0.3	19.2 ± 0.7	8.5 ± 0.3	19.2 ± 0.5
	Ratio	0.98 ± 0.06ab	0.87 ± 0.06b	0.87 ± 0.10b	0.86 ± 0.05b	1.20 ± 0.08ab	1.40 ± 0.05a	1.27 ± 0.13ab	0.94 ± 0.14b
CEC (cMol+ kg^{-1})	0	18.7 ± 0.7	20.9 ± 1.0	19.0 ± 0.8	23.6 ± 2.0	24.7 ± 0.7	33.0 ± 0.7	25.2 ± 0.9	35.5 ± 2.7
	55	19.4 ± 0.7	26.9 ± 1.8	19.0 ± 0.2	25.6 ± 0.3	23.4 ± 0.3	31.2 ± 0.2	23.6 ± 0.3	30.9 ± 0.6
	Ratio	0.97 ± 0.05ab	0.78 ± 0.04b	1.00 ± 0.03a	0.92 ± 0.07ab	1.06 ± 0.03a	1.06 ± 0.03a	1.07 ± 0.04a	1.15 ± 0.07a
Mineral N (mg kg^{-1})	0	1.0 ± 0.2	0.7 ± 0.2	32.8 ± 2.9	19.2 ± 2.3	4.2 ± 0.5	0.6 ± 0.1	14.3 ± 3.4	9.1 ± 2.1
	55	0.8 ± 0.1	0.6 ± 0.2	1.0 ± 0.1	0.6 ± 0.2	1.1 ± 0.1	1.0 ± 0.3	1.1 ± 0.1	1.1 ± 0.2
	Ratio	1.1 ± 0.1de	1.4 ± 0.7cde	34.4 ± 4.0a	38.3 ± 10.4a	3.9 ± 0.7bcd	1.0 ± 0.4e	13.4 ± 3.1ab	9.4 ± 3.2bc
NH_4-N (mg kg^{-1})	0	0.6 ± 0.1	0.7 ± 0.2	11.9 ± 1.4	1.1 ± 0.2	0.3 ± 0.1	0.1 ± 0.1	6.4 ± 2.3	0.7 ± 0.4
	55	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.7 ± 0.2	0.3 ± 0.1	0.2 ± 0.0	0.3 ± 0.1
	Ratio	3.0 ± 0.4	6.6 ± 2.6	123.4 ± 21.4	12.4 ± 1.9	0.7 ± 0.3	0.9 ± 0.7	37.9 ± 11.4	3.1 ± 0.7
NO_3-N (mg kg^{-1})	0	0.4 ± 0.1	ND	20.9 ± 1.5	18.2 ± 2.3	3.9 ± 0.5	0.5 ± 0.1	7.9 ± 1.2	8.4 ± 1.8
	55	0.7 ± 0.1	0.4 ± 0.2	0.9 ± 0.1	0.6 ± 0.2	0.4 ± 0.1	0.7 ± 0.3	0.9 ± 0.1	0.9 ± 0.2
	Ratio	0.5 ± 0.2	--	24.4 ± 2.3	46.1 ± 14.2	19.7 ± 12.5	4.8 ± 4.0	8.9 ± 1.6	12.9 ± 5.0
PO_4-P (mg kg^{-1})	0	5.8 ± 0.1	7.4 ± 0.3	10.5 ± 0.5	11.9 ± 0.5	8.1 ± 0.5	16.1 ± 2.3	7.8 ± 0.3	12.3 ± 2.1
	55	7.1 ± 0.8	8.4 ± 0.4	8.6 ± 0.2	11.6 ± 0.6	6.2 ± 0.3	8.5 ± 0.1	7.1 ± 0.2	9.9 ± 0.3
	Ratio	0.86 ± 0.10b	0.89 ± 0.06b	1.21 ± 0.04ab	1.04 ± 0.07b	1.32 ± 0.16ab	1.92 ± 0.29a	1.11 ± 0.07b	1.23 ± 0.19ab

† Different letters indicate significant differences (p < 0.05) between the cumulative results of treatments (Tukey's correction for multiple testing, n = 4).

Although average total N values for biochar-amended columns were higher than average total N values for their un-amended counterparts, there was no significant difference between soil change ratios for total N. Mineral N exhibited greater differences between treatments than did total N. Although day 55 values for mineral N were relatively similar between treatments (mean values ranged from 0.6 – 1.1 mg kg^{-1}), mean day 0 values ranged from 0.6 – 32.8 mg kg^{-1}. Soil change ratios indicated the largest decreases in mineral N were from fertilizer-amended columns followed by slurry-amended columns. MB columns exhibited a slight increase in mineral N (Table 3). The soil change ratio of mineral N was significantly affected by N source ($p < 0.0001$) and there was an interaction between N source and biochar ($p = 0.0150$). The presence of biochar may have had a weak effect ($p = 0.0593$). The Tukey Test indicated that mineral N soil change ratio for MB was significantly different from that of the M treatment.

Lower NH_4^+ results at day 0 for soils amended with biochar, relative to the corresponding treatments with no biochar (Table 3), indicated that biochar prevented complete extraction of NH_4^+-N from soil. The soil change ratios (which correct for the effect of biochar on the extraction process) suggest that NH_4^+-N lost over the duration of the experiment was lower for columns that had been amended with biochar than those that had not (Table 3). The soil change ratios also indicated that the largest relative decreases in soil PO_4^{3-}-P were in manure-amended columns and that there was a slight increase of PO_4^{3-}-P in control and B columns (Table 3). Presence of biochar, however, had no significant effect on the soil change ratios for PO_4^{3-}-P.

The soil change ratios for K^+ and Mg^{2+} (data not shown) indicated no significant effect of N source or biochar, or of an interaction between N source and biochar. However, there was a significant interaction between N source and biochar on soil change ratios for Ca^{2+}. Over the course of the experiment, Ca^{2+} concentration increased in control, MB and SB columns and decreased in B, F, FB, M, and S columns.

Average CEC values for biochar-amended columns were 2.2–10.4 $cmol^{+}\ kg^{-1}$ higher than average CEC values for their un-amended counterparts at the beginning of the experiment, and 6.6-7.8 $cmol^{+}\ kg^{-1}$ higher than average CEC values for their un-amended counterparts at the end of the experiment. The soil change ratios indicate a slight increase in the mean values of the control, B, F and FB columns and a slight decrease in the mean values of the M, MB, S, and SL columns. The ratio of CEC values was significantly affected by N source (p = 0.0002). Although the CEC ratio was not affected by the presence of biochar (p = 0.1027), there was a significant interaction effect (p = 0.0445).

3.4 Discussion

3.4.1 pH and carbon

The apparent effect of biochar on leachate pH was statistically significant for all treatments at day 0 and day 48 leaching events, indicating that the impact of biochar is immediate but also at least somewhat enduring. Soil pH for B, F, FB, S, and SB samples increased between day 0 and day 55. Soil pH from the control and M columns remained relatively consistent while those of the MB samples decreased. Biochar affected pH of day 0 soil only for samples co-amended with manure, and for both slurry and manure amended soils at day 55. It is likely that pH was initially affected more by slurry and fertilizer addition, and that the liming effect of biochar increased over time as alkaline ash from the char was leached into the soil.

The TOC concentration of the soil used in this experiment was dramatically and enduringly increased by the addition of carbon-rich biochar. Soils that had been amended with manure or slurry decreased in TOC over the duration of the experiment, most likely through microbial respiration of slurry or manure C, as the carbon content of the unamended soil was minimal (Table 1) and as biochar labile carbon content is usually much less than 1% of the total C in biochar (Zimmerman, 2010; Cross and Sohi, 2011).

3.4.2 Nitrogen

In this experiment, application rates of fertilizer, manure and slurry were based on total N and standardized at a rate equal to 66.0 mg total N per column (equivalent to 120 kg N ha^{-1}). Fertilizer N was applied as 100% plant-available (water-soluble) N, whilst slurries tend to have high available N in the time immediately following application (35–70% of total N) and manures tend to have lower available N (10–25% of total N) (DEFRA, 2010). Although the biochar that was used in this experiment included N, the amount of mineral N in the biochar was negligible. Thus, the highest concentrations of mineral N from initial leaching events were from the columns amended with fertilizer. The delay in peak release of mineral N from slurry-amended columns indicates that a pool of slurry N was

mineralized and nitrified during the first few weeks of the experiment. Cumulative N leached from M and MB samples were statistically similar to N leached from control and B samples (respectively), as initial mineral N values of manure amended columns were low. The results for the control, B, and MB treatments indicate that a greater amount of mineral N was present in the leachate than was reflected in the change in mineral N content of the soil samples, indicating that a portion of the mineral N in the leachate must have resulted from mineralization or nitrification in the soil after day 0. The MB treatment, which had the highest average TOC and pH values at day 0, exhibited the greatest decrease in TOC as well as an average increase in soil mineral N, indicating favorable conditions for mineralization of organic matter. F, FB, M, S, and SB treatments exhibited a greater change in mineral N content of the soil samples than was present in leachate, most likely due to gaseous release.

It has been proposed that biochar sorbs ions though a combination of electrostatic and non-electrostatic interactions (Moreno-Castilla, 2004) and also by capillary forces in micropores (Major et al., 2009). Although Cheng et al. (2008) observed that fresh biochar can exhibit net positive charge, soil NO_3^- at the end of the present experiment was not affected by the presence of biochar, either on its own or in combination with fertilizer, manure or slurry. At the same time, mineral N in leachate was lower from biochar amended columns, suggesting that biochar either promoted biotic immobilization, or facilitated the accumulation of NH_4^+ by chemical sorption or the suppression of nitrification. It has been demonstrated that biochar can impact the nitrification process (Clough et al., 2010), possibly due to hydrocarbons sorbed to biochar having an initial effect of suppression of soil microbial communities (Spokas et al., 2009). This may have been reflected in the microbial inhibition that was observed in Spokas and Reicosky (2009). In the present study, an impact on the nitrification process may be reflected in higher NH_4^+ soil change ratios of biochar amended soils and the higher NO_3^- concentrations in leachates from soils that had not been amended with biochar, though other studies have reported no inhibition of nitrification rates (Singh et al., 2010; Bruun et al., 2011). It is also possible that a change in soil pH (reflecting that seen in leachate)

could have had an indirect effect through modification of mineralization rates although, since similar effects have been previously observed in high-pH calcareous sandy soil (Brockhoff et al., 2010), this is less probable. Higher leachate NH_4^+ concentrations for soils that had not been amended with biochar suggest sorption on negatively charged sites within biochar pores.

Consistent with the leaching results in the present study, Laird et al. (2010) reported a decrease of mineral N in leachate from manure-amended soil that had also been amended with biochar. In contrast to the present study, Laird et al. (2010) observed an increase in the mineral N content of the soil after biochar addition but before manure application (the amendments being sequential rather than simultaneous). Laird et al. (2010) proposed that the addition of biochar enhanced mineralization of soil N prior to manure application, but that manure N and soluble organic compounds were bound to the biochar that would otherwise have mineralized or nitrified. Initial soil nutrient content was not reported by Laird et al. (2010), but it is likely that the Midwestern agricultural soil used in their study had considerably higher organic N than the soil used in this experiment. In the present study, there was probably insufficient organic and inorganic N present in the B columns for this effect to be apparent in leachate results, while the rapid mineralization of co-amendments (or addition of fertilizer) in the other biochar columns would have obscured it if it had occurred.

Ordinarily the addition of substrates with high C to N ratio could well inhibit mineralization, especially in a soil with low nutrient status. The addition of biochar in this study measurably and markedly impacted the C to N ratio of whole soil, the biochar-containing columns containing 1.7–2.7 times more TOC than their non-biochar counterparts at day 0 and 1.9–2.3 times at day 55. However, since biochar is comprised mainly of highly stable forms of C, the potential for immobilization to explain decreased N leaching depends on the size of the labile fraction of C (and any associated N) rather than the total C to N ratio. The labile fraction is usually much less than 1% of the total C

in biochar (Zimmerman, 2010; Cross and Sohi, 2011) and is not likely to have a great impact.

The high temperatures of the glasshouse may have increased ammonification and nitrification rates above typical rates for the UK (Myers, 1975). However, as all columns were subjected to the same temperature range in this study, the difference in results observed among the treatments could be assumed to reflect the effects of the soil amendments.

N_2O release from all treatments diminished during and immediately following leaching events, due to the removal of NO_3^- in the leachate. N_2O emissions increased in the periods between leaching events due to mineralization and the reduction of NO_3^- to N_2O. The maintenance of the soils at or near 100% WHC allowed for steady production of N_2O between leaching events, throughout the duration of the experiment.

Proportionally, biochar was most effective at suppressing N_2O emission from soils to which no N was added, in contrast to the findings of Bruun et al. (2011), who found N_2O emissions suppressed from soils amended with biochar and anaerobically digested slurry, but not from soils that had been amended only with biochar. However, the soils used in Bruun et al. (2011) had approx. 3.7 times more N than the soils used in the present experiment and a much lower soil C-to-N ratio (8.7, as compared to 26.8 in the present study), resulting in higher cumulative emissions from all treatments (with the exception of the control) than those from the F treatment in the present study. When the single outlier data point of the present study was removed (see Results above), all biochar treatments emitted less N_2O than non-biochar counterparts, except for the manure treatments (the MB result was lower than the M but, as noted above, the flux was low and the difference not statistically significant). Fertilizer-, slurry-, and manure-amended columns emitted 1.7, 2.8, and 0.6 % of the total N added at the beginning of the experiment (excluding biochar-N), whilst their biochar-amended counterparts emitted 0.7, 1.0, and 0.3%, respectively.

N_2O emission from the manure amended columns (with and without biochar) was less than N_2O release from control columns. As mentioned above, manure contains relatively low levels of immediately-available N and also contributes C which can result in microbial immobilization of N and consequent decrease in N_2O. Had the experiment been extended until the mineralization of manure N was more complete, a statistically significant difference between the M and MB treatments may have emerged, as seen in the comparison of N_2O flux from manure and manure plus biochar amendments on day 414 in Rogovska et al. (2011).

The cause of N_2O suppression after biochar addition is still unknown. A likely contributor is the increase in soil pH that arises from alkalinity associated with soluble ash contained by biochar (Chapter 2), although the results of some studies indicate the changes in soil pH to have limited impact (Yanai et al., 2007; Case et al., 2012). Another proposed explanation (van Zwieten et al., 2010) revolves around an alteration of soil moisture potential and increased O_2 diffusion into the soil. However, Case et al. (2012) corrected for changes in WHC arising from biochar addition and found that the presence of biochar continued to suppress N_2O emissions. Some studies suggest that N_2O emissions are diminished because soluble organic substrates and NH_4^+-N that are sorbed to biochar surfaces are not bioavailable (Singh et al., 2010) and that the decrease in N_2O is at least partly caused by lower N availability after biochar additions (Taghizadeh-Toosi et al., 2011). The capacity of biochar to sorb NH_4^+ is evident in our day 0 soils results. Taghizadeh-Toosi et al. (2011) also speculated that N_2O could be decreased by absorption of NH_3 by biochar but, given the thorough and immediate incorporation of amendments throughout the soil column in this experiment, NH_3 production was likely to have been minimal. It seems certain that N_2O suppression in biochar-amended soils is due to a number of factors, but those which have the largest contribution to this effect in the short-term are likely to be the sorptive properties that might decrease the availability of organic substrates and NH_4^+–N, and the biotic immobilization of N associated with biochar labile C.

3.4.3 Phosphorus

Unlike N, other nutrients (P) differed between treatments according to the composition of the co amendment. Consequently the F columns received more than 4.6 times as much PO_4^{3-}-P as those of the M treatment, and 5.8 times as much as the S. The total amount of PO_4^{3-}–P added to F and FB treatments were not reflected in the day 0 soil results. The decrease in soil pH resulting from fertilizer (reflected in day 0 soil pH results for fertilizer-amended columns as well as the pH of the leachate from fertilizer-amended columns) may have immobilized P as insoluble iron and aluminum phosphates. The average cumulative PO_4^{3-}-P in leachate from all biochar-amended samples (0.22 ± 0.02 kg ha^{-1}) exceeded the average for all samples that did not contain biochar (0.17 ± 0.02 kg ha^{-1}), presumably due to the water-soluble P content of the biochar ash, but the quantities involved were trivial. The soil change ratios for day 0 and day 55 samplings showed that the treatment displaying the highest initial concentrations of PO_4^{3-}–P (namely MB) lost the greatest proportion of PO_4^{3-}-P during the incubation. However, the MB samples were also the only biochar-amended samples in which PO_4^{3-}-P leaching was lower than in the non-biochar counterparts. Lower P leaching from the columns amended with biochar and manure is consistent with the results of Laird et al. (2010), indicating that other properties associated with manure in combination with biochar may affect leaching. As organic amendments are favored in the microbial synthesis of organic P, and as the MB treatment received a higher TOC and PO_4^{3-}-P application than the M, S, and SB treatments, it is likely that a greater amount of P was transformed through microbial synthesis to organic P in the MB treatment (Malik et al., 2012), and this would be reflected in both the decrease in soil PO_4^{3-}-P over the duration of the experiment and the decrease of PO_4^{3-}-P in leachate from this treatment.

3.5 Conclusions

Both N_2O emission and N leaching after slurry and fertilizer addition were lower for the sandy loam soil used in this experiment when co-amended with hardwood biochar. Over a longer timeframe, a similar effect from manure addition may have emerged. Enhanced biotic immobilization and chemical sorption of NH_4^+ resulting from biochar addition could be inferred from the experimental data. The pH of leachates were increased by biochar addition, indicating that for some soils the alkalinity of biochar may be important in influencing soil N as well as P, which leached in small quantities. Further research and methods are needed to assess the sorptive properties of biochar for N (and P) and to scope both the durability and scalability of the effects of biochar on nutrient management, which in this experiment were assessed at high area-based rates.

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3.6 References

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Chapter 4

Paper 3: 'The impact of biochar on gas emissions from liquid dairy manure'

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Abstract

In order to assess the impact of biochar on greenhouse gas and NH_3 emission from cattle slurry in storage, a 107 day incubation study was conducted with a control slurry treatment and four biochar-amended treatments (n = 4). Biochar treatments included three rates of application in which the biochar was thoroughly mixed into the slurry, and one "cap" treatment, in which the biochar was applied to the surface of the slurry. The highest application rate (2:1 slurry-to-biochar by dry weight) treatment and the biochar cap treatment emitted significantly lower CO_2 and N_2O than the other treatments, the biochar cap treatment emitted significantly higher NH_3 than the other treatments, and the 2:1 biochar treatment emitted significantly higher CH_4 than the control. These data indicate that biochar demonstrates potential for greenhouse gas suppression from slurry in long term storage, but does not demonstrate potential for NH_3 suppression from slurry in storage.

4.1 Introduction

The livestock sector is very significant economically and environmentally. Forty percent of worldwide agricultural gross domestic product comes from livestock operations, providing one third of humanity's protein intake and monopolizing more than a 30% of the earth's terrestrial surface (FAO, 2006). The enormous scale of worldwide livestock production has impacted the environment in many ways, not the least of which is through gaseous emissions, including greenhouse gases (GHGs) and ammonia (NH_3). Carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O), which are emitted from livestock manure, contribute to global warming and (in the case of N_2O) to ozone depletion (IPCC, 2007; Skiba et al., 2012). Furthermore, due to the steady increase in global population and the shift of dietary preferences to higher value foods such as meat and milk, global production of meat is expected to more than double and milk production is expected to increase by more than a third by 2050, and greenhouse gas emissions from the livestock sector will continue to increase accordingly (FAO, 2006; Popp et al., 2010). The amount of these gases that will be emitted from livestock systems will be dependent at least partly on how animal manure is produced and managed.

Liquid manure (slurry) is often stored for months at a time prior to land application due to inconvenience of land application or inaccessibility to the field, or due to regulations on timing and quantity of manure application such as the European Union Nitrates Directive (European Commission, 1991). Manure management and treatment systems have demonstrated the potential to significantly impact gaseous emissions (Aneja et al., 2008). Manure management is key in future sustainable livestock production.

In recent years, biochar has been evaluated as a tool for greenhouse gas reduction and carbon sequestration, particularly from soils (Lehmann, 2007). Biochar has proven to be effective at retaining nitrogen in soils (Ding et al., 2010) and to have the potential to reduce N_2O emissions from soil (Zhang et al., 2012a). Biochar has reduced CO_2 emissions from some soils (Spokas et al., 2009; Liu et al., 2011) and increased CO_2

emissions from other soils (Zhang et al., 2012b). Similarly, studies have shown biochar to decrease CH_4 emissions from soil (Liu et al., 2011) or increase the soil's potential as a CH_4 sink in some cases (Zhang et al., 2012b) or reduce a soil's oxidation capacity in others (Spokas et al., 2009). In addition, biochar has reduced NH_3 loss during the composting of chicken manure (Steiner et al., 2010). However, the impact of biochar on gaseous emissions from cattle slurry in storage has not yet been explored. Due to its high surface area and demonstrated ability to impact gaseous emissions from soils, the addition of biochar to slurry could potentially begin affecting GHG emissions even before application to the soil. In addition, co-application of slurry and biochar could potentially address the logistical challenge of biochar application to soil due to its physical properties, including its inconsistent particle size and the presence of charcoal fines or dust (Shackley and Sohi, 2010).

The purpose of this study was to: (i) examine the impact of fresh biochar applied and thoroughly mixed into slurry at three different application rates on GHG and NH_3 emissions from slurry in long term storage, and (ii) examine the effectiveness of biochar applied as a cap in inhibiting GHG and NH_3 emissions from slurry in long term storage.

4.2 Materials and methods

The study was conducted at Rothamsted Research, North Wyke, in Devon, UK. Dairy cattle slurry was obtained from the slurry store of a commercial dairy farm and stored below 4°C. The biochar was made from willow chips (*Salix* spp.) heated from ambient temperature (8°C) and pressure to 450°C over the course of 80 min and maintained at 450°C for 80 min using a batch process slow pyrolysis unit with a water-cooled condenser, an internal auger for feedstock agitation during the reaction process, and an internal thermocouple (measuring the temperature in the core of the combustion chamber) which controls the process. Biochar and slurry characteristics are presented as Table 1; laboratory methodology is described in 4.2.1.

Table 1 Characteristics of the slurry and biochar used in the experiment (n = 3)

Characteristics	Slurry	Biochar
pH	8	9.3
Dry Matter / mg kg^{-1}	86,000	995,000
$NH4^{+}$-N / mg kg^{-1} (dm)	15,400	--[a]
Total N / mg kg^{-1} (dm)	40,600	8110
TOC (LOI) / mg kg^{-1} (dm)	787,000	935,000
Organic C / mg kg^{-1} (dm)	380,600	542,000
CEC / meq per 100 g	--	22.2

[a] -- Not Analysed

24 h before the start of the experiment, the slurry was thoroughly mixed. 1.5 L of slurry was then poured into each of twenty 3 L glass jars. When filled with 1.5 L of slurry, there was approx. 16 cm height of headspace between the surface of the slurry and the lid of the container. The jars were stored with the lids partially open at 15°C overnight.

24 h after the slurry was added to the jars, biochar was added to the appropriate treatments. There were five treatments with four replicate jars per treatment. Treatments included an unamended control slurry and four biochar amended treatments (biochar application rates for all treatments were determined by mass on a dry weight basis)

including: 1) slurry with biochar added at a 2:1 slurry to biochar ratio; 2) slurry with biochar added at a 10:1 slurry to biochar ratio; 3) slurry with biochar added at a 20:1 slurry to biochar ratio; and 4) slurry with biochar "cap" applied to the surface of the slurry, with the mass of biochar applied equal to that of the 10:1 slurry to biochar ratio treatment. All samples were thoroughly stirred; the biochar cap treatment was stirred prior to biochar application whilst the other biochar treatments were stirred after biochar application.

Jars were incubated in an incubation room in the dark at 15°C for 107 days with the lids partially open to simulate open air storage conditions whilst partly limiting escape of moisture. Lids were only closed during sampling events, as described below.

4.2.1 Slurry sampling and analysis

A slurry sample was taken at the beginning of the study. The sample was stored below 4°C prior to analysis for dry matter (DM), pH, total nitrogen (TN), ammonium (NH_4^+), total carbon, and organic carbon. Biochar was analysed for DM, pH, TN, total carbon, organic carbon, and cation exchange capacity (CEC). DM content was determined gravimetrically by drying a sub-sample in an oven at 105º for 24 h. Total nitrogen was determined by Kjeldahl digestion (Schumacher et al., 1997). Total carbon was determined by loss on ignition (Nelson and Sommers, 1996). CEC was determined according to MAFF (1986). At the end of the study, the crust and slurry were separated in order to determine DM content of each portion, and a representative crust and slurry sample was collected from each jar and analysed for pH, NH_4^+, and nitrate (NO_3^-). DM content was determined gravimetrically by drying a sub-sample in an oven at 105º for 24 h. NH_4^+ and NO_3^- were determined by KCl extraction followed by discrete photometric analysis (Aquakem 250, Thermo Fisher Scientific, Waltham, MA, USA).

All samples included blanks and quality control standards (generally one for every ten samples). Records were kept for each analysis performed and data was not released until the results from the samples included had been verified as correct. The pH meter was

regularly calibrated and the details logged using standards of pH 6.8 and 4.43 to verify that the meter was correctly calibrated.

4.2.2 Analysis of CO_2, N_2O and CH_4

Gas samples were collected on days 1-18, 20-23, 25, 27, 29, 31-40, 42, 44, 47, 49, 51, 54, 56, 70, 77, 84, and 107. Containers were sealed using a glass lid with a rubber seal and metal clamp. Gas samples were collected from a sampling port fixed in the lid of each container with a 50 mL syringe and hypodermic needle. Samples were collected at 0 min, 15 min, and 30 min following the closure of each container. As headspace volumes varied over the course of the experiment, 150 mL of gas samples was the equivalent of approx. 5.7-18.2% of the headspace. Samples were injected into pre-evacuated 20-22 mL headspace vials using a second needle to allow the sample to reach atmospheric pressure. The glass vials were sealed with chloro butyl rubber septums and crimp caps (Chromacol, Welwyn Garden City, Herts, UK) and pre-evacuated using a vacuum pump. The samples were analysed as soon as possible after collection (usually within 24 h) by gas chromatography.

The analysis was performed on a Clarus 580 PE AutoSystem GC (Perkin Elmer, Waltham, MA, USA) with an electron capture detector (ECD) and flame ionisation detector (FID). The separation column was a Perkin Elmer EtileQ PLOT megabore capillary (30 m × 0.53 mm i.d.) which was operated at 30°C. The ECD detector was set at 375°C and the carrier gas was N_2. The retention times for CH_4, N_2O, and CO_2 were 0.96 min, 1.28 min, and 1.25 min, respectively. The total time for one sample was set to 4.50 min. One quality control standard was included for every ten samples. N_2O standards were 0.3, 1.6, 5.0, and 49 ppm; CH_4 standards were 2.2, 5.2, 9.8, 50, and 100 ppm; and CO_2 standards were 500 and 2500 ppm.

4.2.3 Analysis of NH_3

Emission of NH_3 was estimated using the method described by Dendooven et al., (1998). Acid traps were prepared using 10 mL of 0.1 M boric acid. The traps were fixed to the

inside of each container prior to closure of the lid, and were collected after 4 h (Figure 1). Samples were taken on days 1, 2, 3, 4, 5, 7, 9, 12, 14, 22, and 84. The aqueous NH_4^+-N concentration of these traps was determined by discrete photometric analysis (Aquakem 250, Thermo Fisher Scientific, Waltham, MA, USA). The limit of detection was 0.1 mg NH_4^+-N L^{-1} and the working range was 0.4-250 mg NH_4^+-N L^{-1}.

Figure 1: Photograph of slurry in storage during a NH_3 sampling event when lids were closed for 4 hours

4.2.4 Data Analysis

Cumulative gas emissions were estimated for each replicate using the linear trapezoidal method. Emissions are presented as g m^{-2}. GHG emission as CO_2 equivalent (CO_2e) was determined by assigning global warming potential values of 1 for CO_2, 25 for CH_4, and 298 for N_2O (Forster et al., 2007). Treatment effects on cumulative emissions, pH, TAN,

NO_3^-, and mass remaining on day 107 were assessed using one-way analysis of variance (ANOVA). Cumulative emissions, pH, NH_4^+, and NO_3^- values were log-transformed (natural log) prior to statistical analysis in order to achieve normality. Post hoc comparisons were performed using Tukey's correction for multiple testing. Model fit was assessed using graphical analysis of residuals, a Shapiro-Wilk test for normality, and the O'Brien test for equal variance. All results were normally distributed unless otherwise noted below. The 0.05 level was used to determine statistical significance. All statistical analyses were conducted using JMP 9.0.0 (SAS Institute, Cary, NC, USA).

4.3 Results and discussion

Results from 10:1 and 20:1 ratio slurry-to-biochar treatments were not significantly different from the control for any of the parameters measured. This indicates that these rates of biochar application (when thoroughly mixed into the slurry) were not high enough to significantly affect gaseous emissions or nutrient dynamics within stored slurry. Given that the 10:1 ratio biochar treatment and the cap treatment contained the same amount of biochar and given that there were significant treatment effects for the cap treatment, this also indicates that the location of the biochar with respect to the slurry is as important as the amount of biochar applied.

4.3.1 Change in mass

Remaining mass (on a fresh weight basis) for the biochar cap treatment was significantly higher (55.4%) than the control (46.9%), whilst the remaining mass (fresh weight) of the 2:1 biochar treatment was not significantly different from the control. On the other hand, remaining mass (on a dry weight basis) of the 2:1 ratio biochar treatment (97.4%) was significantly higher than the control (88.3%) whereas the remaining mass (dry weight basis) of the biochar cap treatment was not significantly different from the control (Table 2). This indicates that the biochar cap treatment aided in the formation of a barrier that was effective in prohibiting the movement of water vapour through the crust of the slurry, perhaps due to the hydrophobicity of fresh biochar (Lehmann et al., 2009), whilst the 2:1 ratio biochar treatment was more effective at limiting loss of mass through microbial activity. However, these (dry weight) results likely reflect the high biochar content which was included in the initial mass measurement of the 2:1 ratio treatment which is more stable and resistant to microbial degradation than the slurry.

Table 2 Analysis of slurry and crust at the end of the experiment, means and standard errors (n = 4)

		Control	Slurry + biochar (2:1 ratio)	Slurry + biochar (10:1 ratio)	Slurry + biochar (20:1 ratio)	Slurry + biochar (10:1 ratio "cap")
Slurry						
Wt Remaining	g (dw)	97.8 (2.5)	172.5 (6.4)	103.9 (2.0)	102.0 (4.7)	118.8 (5.1)
NH_4-N	mg kg^{-1}	192 (49)ab	148 (13)b	239 (101)ab	356 (125)ab	599 (45)a
NO_3-N	mg kg^{-1}	7.5 (7.5)b	41.2 (8.9)a	13.3 (11.4)ab	3.4 (3.4)b	NDb
pH		9.0 (0.1)a	8.9 (0.2)a	9.0 (0.1)a	8.9 (0.1)a	8.8 (0.1)a
Crust						
Wt Remaining	g (dw)	20.8 (1.1)	28.0 (3.0)	27.5 (3.2)	27.6 (2.0)	23.3 (0.4)
NH_4-N	mg kg^{-1}	145 (66)a	70 (16)a	88 (21)a	184 (73)a	39 (16)a
NO_3-N	mg kg^{-1}	1270 (255)ab	1277 (190)ab	1468 (227)ab	1899 (364)a	665 (150)b
Initial Weights						
Fresh Weight	g	1548 (23)	1651 (27)	1557 (3)	1590 (27)	1572 (7)
Dry Weight	g	134 (2)	206 (3)	147 (0)	144 (2)	149 (1)
Remaining Crust + Slurry (end of experiment)						
Fresh Weight	%	46.9 (1.3)b	53.2 (1.3)ab	45.7 (2.9)b	47.1 (2.2)b	55.4 (1.0)a
Dry Weight	%	88.3 (1.8)b	97.4 (0.9)a	89.3 (0.7)b	89.9 (1.6)ab	95.4 (2.8)ab

ND Results were below detection limits

Different superscripts indicate significant differences at $p < 0.05$

Values in parentheses are standard errors of the mean

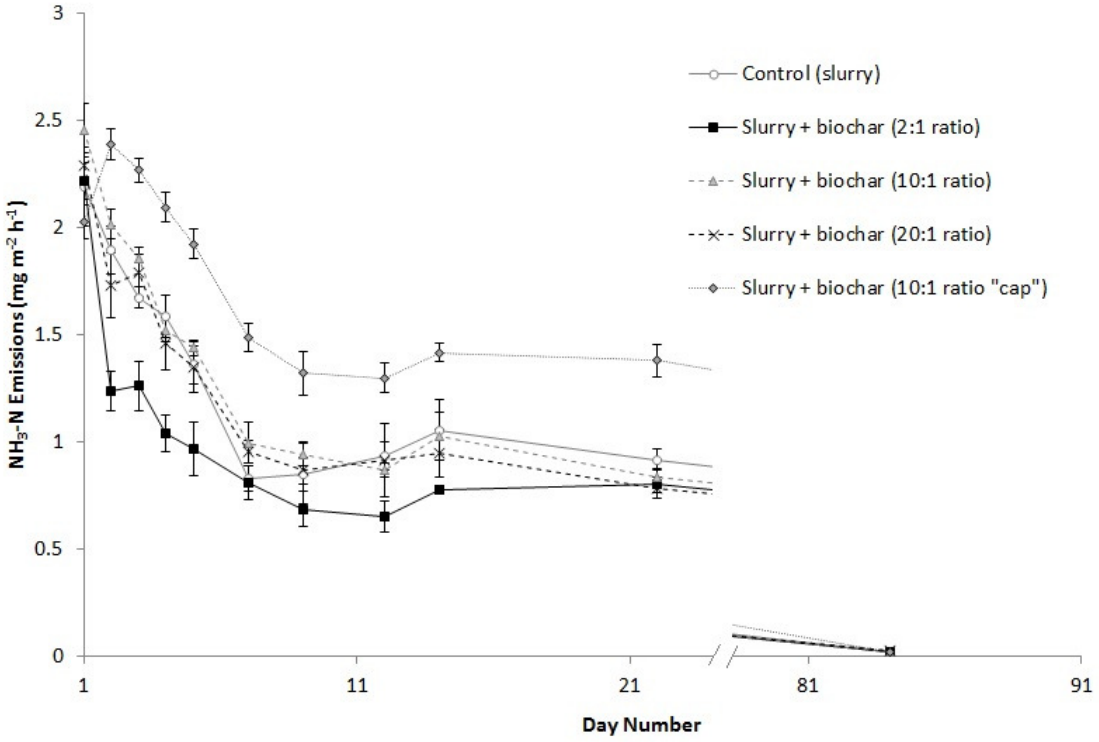
4.3.2 Ammonia emission

Cumulative NH_3 results (Table 3) were within the range of those demonstrated in the slurry incubation experiment performed by Amon et al. (2006), but much lower than those demonstrated in Wood et al. (2012). Highest average NH_3-N emission from the biochar cap treatment occurred on day 2, whilst highest average NH_3-N emission from all other treatments occurred on day 1. With the exception of a slight increase in average emission between day 2 and day 3 from the 2:1 ratio treatment, average emission from all treatments decreased steadily between day 2 and day 7, most likely due to crust formation. There were increases in average emission rates from all treatments over one or more sampling events between day 9 and day 22. NH_3-N was below the detection limit for all jars on day 84, with the exception of one jar (20:1 ratio) which indicated an emission rate of 0.05 mg NH_3-N m^{-2} h^{-1} (Fig. 1). ANOVA results indicate a significant treatment effect ($p < 0.0001$), though the only treatment that demonstrated cumulative emissions that were significantly different from the control was the biochar cap treatment, from which NH_3-N emissions were significantly higher than the other treatments (1.83 g m^{-2}).

Although the biochar cap treatment demonstrated the lowest average NH_3-N emission rate on day 1, NH_3-N emission from this treatment was higher than all other treatments for all subsequent sampling events with the exception of day 84. The increase of NH_3-N emission from the biochar cap treatment may have been the result of an increase in pH due to the high concentration of biochar at the surface of the slurry, though the pH of the surface of the slurry was not measured. Although there was no significant difference between treatments for slurry pH at the end of the experiment (Table 2), biochar has been shown to have a liming effect (Chan et al., 2007; Liu et al., 2012; Zhang et al., 2012a), which affects the production of NH_3. The biochar cap which was approx. 2 cm in thickness was not as thick as the typical recommendations (15-30 cm) for a straw cap (VanderZaag et al., 2009), so it was not sufficient to act as a physical barrier for NH_3. Furthermore, cracks formed in the cap, allowing the slurry (and NH_3) to move through toward the surface. This may have increased the surface area of the slurry that was

exposed to the atmosphere, thereby increasing NH_3 emission. Also, straw caps have occasionally been shown to increase NH_3 emission due to the increase in dry matter at the surface (Amon et al., 2006) and the same could potentially occur for a biochar cap.

Figure 1: Effect of biochar on NH_3-N emissions from slurry, means and standard errors ($n = 4$)



The 2:1 ratio biochar treatment demonstrated the lowest NH_3-N emission for each sampling event from day 2 until day 14. Average cumulative emissions from the 2:1 ratio biochar treatment were lower than all other treatments (1.05 g NH_3-N m^{-2}), though not significantly (Table 3). The decrease in NH_3 emissions from the 2:1 ratio samples could be due to the N adsorbing to the biochar as with zeolite, the higher C:N ratio, and/or direct absorption of the gas into biochar pores as with sphagnum peat (McCrory and Hobbs, 2001). The potential may still exist for the use of biochar for NH_3 suppression, though perhaps not at a practical application rate, or perhaps with biochar made from a different

feedstock or made under different process conditions than the biochar that was used in this experiment. For instance, an acidified pine chip or pelletized peanut hull biochar produced at a lower temperature may have suppressed NH_3 emission (Doydora et al., 2011).

Table 3 Net total gaseous emissions during storage, means and standard errors (n = 4)

Treatment	CO_2-C	EF[a]	CH_4-C	EF	N_2O-N	EF	NH_3-N	EF	CO_2 Equivalent
	$g\ m^{-2}$	%	$g\ m^{-2}$	%	$g\ m^{-2}$	%	$g\ m^{-2}$	%	$g\ m^{-2}$
Control	883 (24)a	10.3 (0.3)	65 (5)b	0.75 (0.06)	12.7 (0.8)a	2.9 (0.2)	1.26 (0.06)b	0.28 (0.06)	11,347 (456)a
Slurry + Biochar (2:1 ratio)	505 (30)b	3.6 (0.2)	99 (11)a	0.71 (0.08)	4.2 (0.5)b	0.8 (0.1)	1.05 (0.04)b	0.21 (0.01)	7115 (564)b
Slurry + Biochar (10:1 ratio)	842 (19)a	8.8 (0.2)	74 (5)ab	0.77 (0.05)	10.0 (1.0)a	2.2 (0.2)	1.20 (0.03)b	0.27 (0.01)	10,220 (612)a
Slurry + Biochar (20:1 ratio)	935 (11)a	10.0 (0.2)	69 (6)ab	0.74 (0.07)	12.5 (1.0)a	2.7 (0.2)	1.14 (0.06)b	0.25 (0.01)	11,589 (690)a
Slurry + Biochar (10:1 ratio "cap")	539 (36)b	5.6 (0.4)	90 (10)ab	0.93 (0.10)	5.9 (0.5)b	1.3 (0.1)	1.83 (0.07)a	0.40 (0.01)	7734 (422)b

Different superscripts indicate signficant differences at $p < 0.05$

Values in parentheses are standard errors of the mean

[a] Emission Factor (% of total C or N)

4.3.3 Ammonium and nitrate

The cap treatment demonstrated significantly higher concentrations of slurry NH_4^+-N (599 mg kg^{-1}) than the other treatments and the lowest average NO_3^--N concentration in both crust (665 mg kg^{-1}) and slurry (all samples were below the detection limit) (Table 2), indicating reduced depletion of the NH_4^+-N pool due to treatment effects on the nitrification, immobilisation, and volatilisation processes. Additionally, the high emission of NH_3-N from this treatment may have inhibited the nitrite oxidizers (Villaverde et al., 1997; Clough et al., 2010). NH_4^+ may have been directly adsorbed to char, or adsorption of NH_3-N by char (Asada et al., 2006) may have been transformed into slurry NH_4^+.

Average slurry NO_3^--N concentrations from the 2:1 biochar treatment (41.2 mg kg^{-1}) were significantly higher than those of the control (7.5 mg kg^{-1}) , and this treatment also demonstrated the lowest slurry NH_4^+-N concentrations (148 mg kg^{-1}), indicating that nitrification rates may have been enhanced rather than inhibited. However, fresh biochar has demonstrated a positive charge (Cheng et al., 2008), and has been shown to sorb NO_3^--N (Mizuta et al., 2004). It is possible that (either due to the sorptive qualities of the biochar or the increased C:N ratio of this treatment) this NO_3^- was not available to denitrifying bacteria.

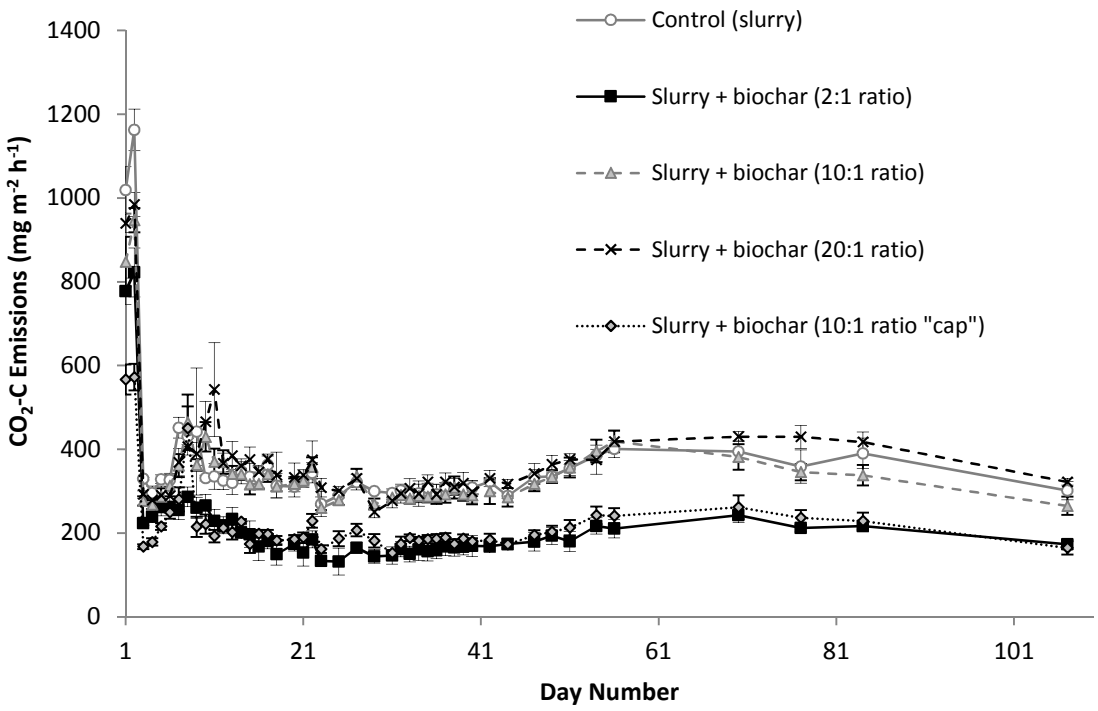
Due to the high number of slurry NO_3^--N results that fell below detection limits, these data failed the normality test. There was no significant difference between treatments for crust NH_4^+-N concentration.

4.3.4 Greenhouse gas emission

CO_2-C fluxes were highly variable over the first 11 days of sampling, but from days 12-107 the control, 10:1 ratio, and 20:1 ratio all maintained rates of emission approx. 300-700 mg m^{-2} h^{-1} higher than those of 2:1 ratio or the biochar cap treatments (Fig. 2). Average cumulative results for the control, 10:1 ratio and 20:1 ratio ranged from 842-935

g CO_2-C m^{-2} slurry (Table 3), whilst average cumulative results from the biochar cap treatment and the 2:1 biochar treatment were 505 and 539 g m^{-2}, respectively. These emissions represented approx. 3.6-10.3% of the total slurry C present at the beginning of the experiment. ANOVA results for cumulative CO_2-C emissions indicate a significant treatment effect ($p < 0.0001$) with the 2:1 biochar treatment and the biochar cap treatment demonstrating significantly lower emissions than the control, the 10:1 biochar treatment, or the 20:1 biochar treatment.

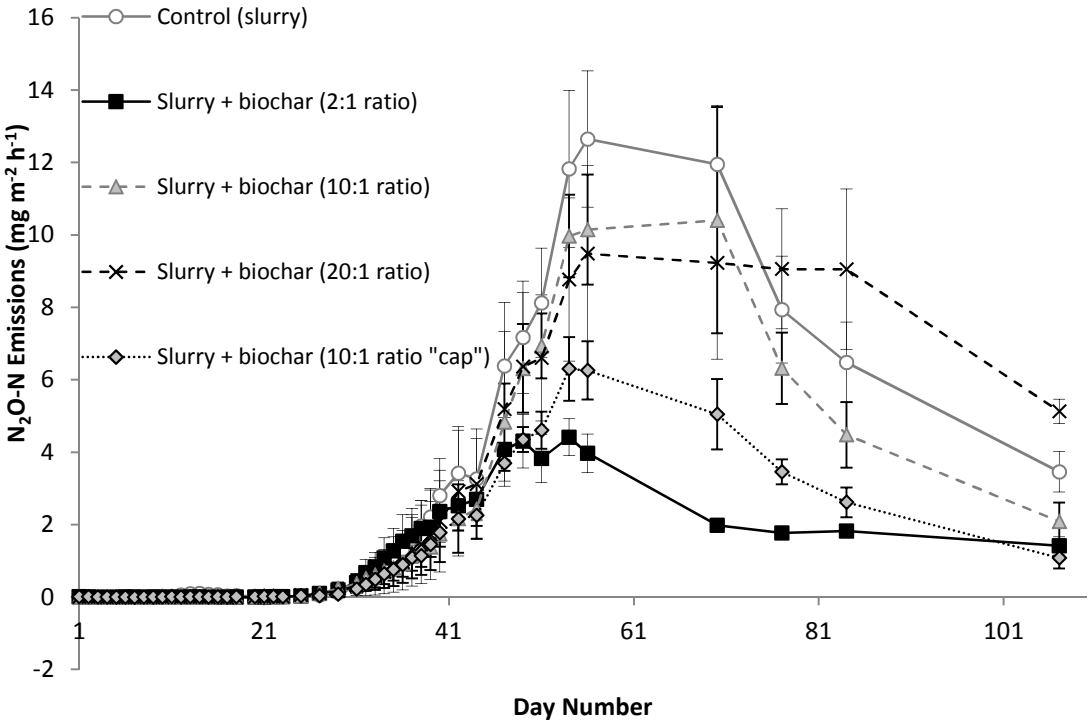
Figure 2: Effect of biochar on CO_2-C emissions from slurry, means and standard errors ($n = 4$)



There were no N_2O-N fluxes for the first 36 days of the experiment. Highest N_2O-N emissions in most treatments occurred between day 54 and day 70, including the control, the 1:10 ratio treatment, and the biochar cap treatment. Highest N_2O emissions from 3 of the 4 2:1 ratio treatment jars occurred earlier, at day 41, day 47, and day 49, respectively. Highest emissions from one 20:1 ratio treatment jar occurred later, at day

84 (Fig. 3). Average N_2O-N emissions ranged from 4.2 g m^{-2} (2:1 ratio biochar treatment) to 12.7 g m^{-2} (control) (Table 3). Based on these averages, approximately 2.9% of the total N originally present in the slurry was emitted as N_2O from the control treatments and 0.8% from the 2:1 ratio treatment. ANOVA results indicate a significant treatment effect ($p < 0.0001$) with the 2:1 biochar treatment and the biochar cap treatment demonstrating significantly lower emissions than the control, the 10:1 biochar treatment, or the 20:1 biochar treatment.

Figure 3: Effect of biochar on N_2O-N emissions from slurry, means and standard errors ($n = 4$)



Hourly rates of emission of N_2O-N were within the range of those demonstrated by Sommer et al. (2000). Cumulative N_2O results (on an area basis) were similar to those demonstrated by Wood et al. (2012), but (when converted to a volume basis) were 4.5-19.3 times higher at day 77 than the 80 day cumulative results of the control treatment in

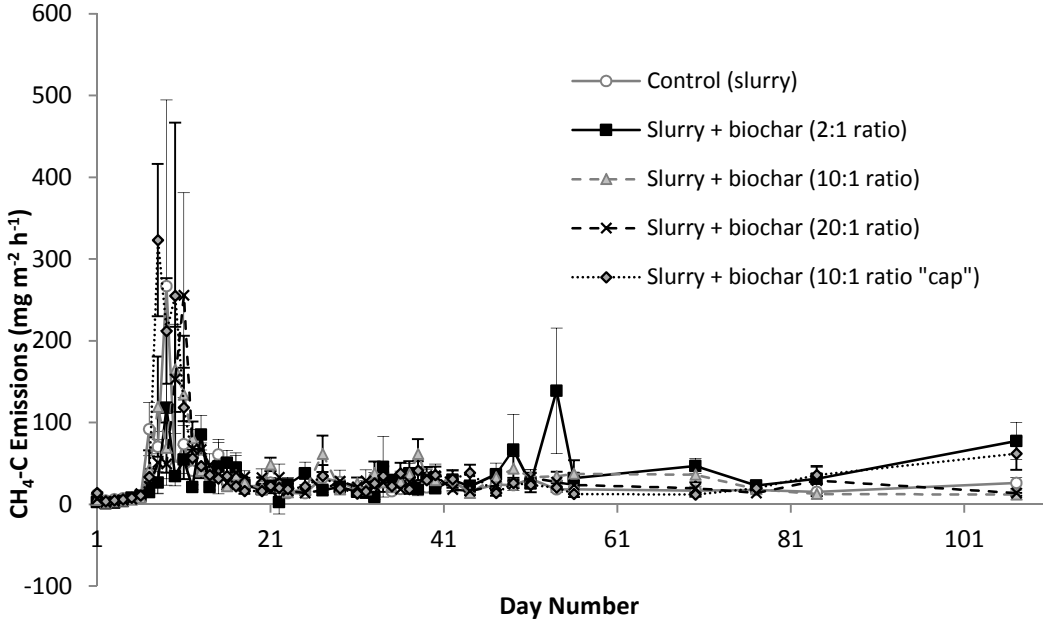
Amon et al. (2006). Wood et al. (2012) and Amon et al. (2006) both utilised through-flow systems rather than static chambers with manure depths of approx. 1.6 m and 2.0 m, respectively. Sommer et al. (2000) utilised a closed chamber method with a manure depth of approx. 1.4 m. Cumulative gas emissions from the present study may have been affected by the volume of slurry, which was smaller than any of the above referenced studies, allowing diffusion of O_2 to a greater proportion of the slurry and enhancing nitrification rates. Nitrate data was not available for any of the above referenced studies, but NH_4^+ concentrations in the slurry of the Wood et al. (2012) study were much higher than those in the present study. Additionally, high N_2O fluxes may have been the result of removing ca. 5.7-18.2% (depending on day and treatment type) of the headspace volume in the three gas samples taken on each sampling occasion, which could have drawn gas from within the slurry crust.

The mechanisms for N_2O suppression from the biochar cap treatment may have been different from the mechanisms for N_2O suppression from the 2:1 ratio biochar treatment. Sommer et al. (2000) observed a decrease in N_2O emissions from cattle slurry with a straw cover, however this effect was presumed to be related to water balance from rainfall which is not a factor in the present experiment. The decrease in N_2O emissions from the biochar cap treatment may have been the result of an increase in pH due to the high concentration of biochar at the surface of the slurry. As the crust pH was not assessed at the end of the experiment, pH results in Table 2 do not reflect the pH at the surface of the slurry. The decrease in N_2O emissions may have been partly due to a decrease in mineral N following the heightened NH_3 release (Table 3), but given that only a small quantity of N is released as NH_3 (<2%), this could not be the dominant mechanism of suppression. N_2O suppression was not likely due to NH_4^+ adsorbing to the biochar, as the biochar cap treatment exhibited an increase in NH_3 emissions in comparison with the control. Additionally, the low rate of N_2O emission from this treatment is most likely due in part to the low concentration of NO_3^--N found in the slurry at the end of the experiment (Table 2). Conversely, the high concentration of NO_3^--N in the 2:1 biochar treatment indicates that N_2O suppression from this treatment is possibly due to a combination of a temporary

change in pH that was not significant at the end of the experiment, or to the sorption of mineral N to biochar surfaces (see above).

Highest CH_4-C emissions in most treatments occurred between day 8 and day 11 with the exception of two of the 2:1 ratio treatment jars for which the highest emissions occurred at day 49 and day 54, respectively. There was also an increase in average CH_4-C emissions from 2:1 ratio and biochar cap treatments between day 84 and day 107 (Fig. 4). ANOVA results for cumulative CH_4-C emissions indicated a significant treatment effect (p = 0.0227), though the only treatments for which the means were significantly different from one another were the control (65 g m^{-2}) and the 2:1 ratio biochar treatment (99 g m^{-2}) (Table 3). Average CH_4-C emissions from the biochar cap treatment were also higher than those of the control, but the difference between mean values was not significant. Cumulative CH_4 results were within the range of those demonstrated in the slurry incubation experiment performed by Amon et al. (2006), but much lower than those demonstrated in Wood et al. (2012). Maximum hourly rates of emission from two of the treatments were within the range reported by Sommer et al. (2000), but maximum rates of three of the treatments in the present study exceeded those reported by Sommer et al. (2000) by approx. 27-47%.

Figure 4: Effect of biochar on CH_4-C emissions from slurry, means and standard errors ($n = 4$)



CH_4 emissions were initially suppressed in the 2:1 ratio treatment samples, possibly due to temporarily increased aeration due to the higher porosity of the biochar and resulting increase to the methanotroph population (Petersen et al., 2005). Another possible explanation is that temporary CH_4 suppression (as well as, potentially, suppression of CO_2 and N_2O from this treatment) was due to organics sorbed to the biochar that may have been toxic to microbiota in the slurry. There has been some indication that biochar suppresses microbial activities initially, but the long term effects are unknown (Spokas et al., 2009). Subsequent CH_4 peaks from the 2:1 ratio treatment, however, resulted in cumulative results that were significantly higher than the control. The later peak emissions from the 2:1 ratio biochar treatment may have occurred when new pools of less degradable substrates became available for methanogens upon prolonged storage (due to microbial community changes), or it may be that there was a lag before the methanogen population established itself. If the relative increase in CH_4 release from 2:1 treatment was due to the degradation of labile carbon, this could explain the slight (though not statistically significant) suppression of NH_3 release from this treatment relative to the control (McCrory and Hobbs, 2001). The porosity of the biochar and the resulting crust

may have also aided in the rapid movement of CH_4 through the crust, thereby limiting exposure to potential methanotrophs and acting as the opposite of a cap (Petersen et al., 2005). It is also possible that crust formation was impaired in the 2:1 ratio biochar treatment due to the greater viscosity and higher drag forces of the slurry/biochar mixture.

The slight increase in CH_4-C from the biochar cap treatment could be due to a small amount of labile carbon in the biochar, but that would probably have resulted in a higher CO_2 flux as well (which did not occur). Likewise, if the cap decreased aeration and facilitated anaerobic conditions, this would have increased the CH_4 flux (Amon et al., 2006), but would have also increased the CO_2 flux, which it did not. CO_2 and CH_4 are both low-solubility gases which move across the aqueous boundary by both diffusion and ebullition. If the presence of biochar in any way physically inhibited the movement of CO_2 through the boundary, the same would have occurred for CH_4. In addition, covers often have no impact on CH_4 emission as "plumes" generated through ebullition (and the large concentration gradient across the cover) minimize the effectiveness of the cover (Hudson et al., 2006). A localised increase in pH at the surface of the slurry (which was not assessed in this experiment) could also potentially explain an increase in CH_4 (Petersen et al., 2012).

The relatively higher initial gaseous production and ebullition in the control, 10:1 ratio biochar, and 20:1 ratio biochar treatments, paired with a comparatively lower total solids content (in comparison with the 2:1 ratio biochar treatment) may have led to faster crust formation in these treatments (Sommer et al., 2007; Wood et al., 2012), which in turn would (later in the experiment) slow CH_4 transport via ebullition and facilitate oxidation. An increased oxidation rate would also explain the higher CO_2 fluxes in these treatments, as compared to the 2:1 ratio biochar and biochar cap treatments. In the control, 10:1 ratio, and 20:1 ratio biochar treatments, methanogenesis is slow in comparison with other degradation pathways producing CO_2, which include fermentation processes and aerobic microbial activity at the slurry-air interface (Møller et al., 2004).

Based on these values, average CO_2-equivalent (CO_2e) emissions from the control containers exceeded all other treatments with cumulative emissions of 11,347 g CO_2e m^{-2}. Average CO_2e emissions from the 2:1 ratio treatment were less than all other treatments with cumulative emissions of 7115 g CO_2e m^{-2} (Table 3). ANOVA results indicate a significant treatment effect ($p < 0.0001$) with the 2:1 biochar treatment and the biochar cap treatment demonstrating significantly lower CO_2e emissions than the control, the 10:1 biochar treatment, or the 20:1 biochar treatment. As average N_2O emissions were at their peak between day 54 and day 70, and as N_2O has the highest global warming potential of the three GHGs measured in this study, biochar demonstrates the strongest potential for GHG (CO_2e) suppression in situations of long term (> 30 days) storage.

While effective in GHG mitigation, the 2:1 ratio biochar application rate is not a practical application rate for a farm-scale manure pit or tank. It is apparent that biochar used as a cap has the potential to significantly decrease GHG emissions from long-term storage of slurry, though NH_3 emissions may increase from slurry that is stored in this manner.

4.4 Conclusions

Biochar that has been mixed into slurry at a 10:1 or 20:1 slurry to biochar ratio is not at a high enough application rate to significantly affect gaseous emissions from slurry. The 2:1 ratio treatment demonstrated cumulative CH_4 emissions that were significantly higher than those of the control, and the biochar cap treatment exhibited cumulative NH_3 emissions that were higher than all other treatments. The 2:1 ratio biochar treatment and the biochar cap treatment both demonstrated potential to inhibit N_2O and CO_2 emissions, and therefore had significantly lower average cumulative CO_2e emissions than the other treatments. Although care must be taken in extrapolating laboratory scale incubation results to estimate emissions from larger scale slurry storage, it is apparent that biochar has the potential to significantly decrease GHG emissions from long-term storage of slurry, but (when used at a practical application rate) does not appear to be effective in reducing NH_3 emissions from slurry in storage.

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Chapter 5

Paper 4: 'Impact of pine chip biochar on trace greenhouse gas emissions and soil nutrient dynamics in an annual ryegrass system in California'

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Abstract

Manure generated by dairy cattle is a useful soil amendment but contributes to greenhouse gas (GHG) emissions and water pollution from nutrient leaching. In order to assess the impact of biochar integrated in a dairy grassland system, a one year field-scale study was conducted on a sandy loam soil used to grow annual ryegrasses (*Lolium multiflorum*) for silage in Petaluma, California. Manure was applied to all plots at a rate of approx. 150 m^3 ha^{-1} (410 kg N ha^{-1}). Control plots received no biochar, high application biochar plots (HB) received a pine chip biochar (with a 17% ash content) at a rate of 18.8 t ha^{-1}, and low application biochar plots (LB) received the same biochar at a rate of 5.7 t ha^{-1}. There were four replicates for each treatment. Although the HB plots demonstrated the lowest cumulative nitrous oxide (N_2O), methane (CH_4), and ammonia (NH_3) emissions, there was no significant difference between treatments. Soil pH and total carbon significantly increased in HB plots, and nitrate (NO_3^-) intensity (which expresses potential exposure of NO_3^- to the soil microbial community) significantly decreased in HB plots compared to the control. Nutrient loss in leachate measured using ion exchange resins indicated that annual cumulative potassium (K^+) loss from HB plots was significantly higher than the other treatments. HB plots also demonstrated a short term increase in phosphorus (P) and ammonium (NH_4^+) loss as well as a short term decrease in NO_3^- loss, though differences between treatments for cumulative results for these nutrients were not significant. The preceding data indicate that some types of biochar (particularly those with high ash content) should be used with caution in systems that include high rates of nutrient application if there is a risk of nutrient loading to surface- or groundwater, unless the ash is removed from the char prior to soil application.

5.1 Introduction

Large scale livestock production is considered an important contributor to global greenhouse gas (GHG) emissions and water pollution from nutrient leaching and runoff (FAO, 2006). Global livestock production has rapidly increased in recent decades due to global population growth and changes in human diet, leading to a large increase in manure production (FAO, 2006; Oenema et al., 2005; Oenema et al., 2007; Steinfeld and Wassenaar, 2007). Land application of manure is a waste management practice for dairy systems that can deliver crop nutrients back to soil. However, due to the low density of nutrient value in manure, cost of transport more than a short distance exceeds the nutrient value of the manure. Thus, the land area to which the manure can reasonably be added is limited to a short radius from the point of production (DEFRA, 2004; Sharpley et al., 1994), which frequently includes fields in which grasses are grown for silage or grazing.

The addition of nutrients to soil in excess of that which the crops can use can result in nutrient leaching, which is an important source of water pollution (Stout et al., 2000). High levels of nitrogen may damage ecological systems (Vitousek, 1997) and contaminate groundwater (Spalding and Exner, 1993). If manure is applied at a rate sufficient for crop N requirements, rapid accumulation of soil phosphorus (P) is inevitable, which is likely to result in P leaching and runoff (Olson et al., 2010). Freshwater systems are highly sensitive to pollution by P (Carpenter and Bennett, 2011), which is a limiting nutrient for algal growth and eutrophication (Carpenter, 2008; Schindler et al., 2008). Also, as K^+ requirements in agriculture are second only to nitrogen (N), effective K^+ management is important (Askegaard et al., 2004).

Management of manure is also important for GHG emission control (Oenema et al., 2007). It has been estimated that 2.0% of manure N has been emitted to the atmosphere as nitrous oxide (N_2O) since 1860 (Davidson, 2009), and N_2O fluxes from manure-amended soil can be greater in magnitude and extend over a longer period of time than N_2O fluxes from mineral fertilizer (Jones et al., 2007; Senbayram et al., 2009). Intensive

manure application can also result in substantial amounts of methane (CH_4) emission (Chadwick and Pain, 1997; Chadwick et al., 2000; Rodhe et al., 2006) and ammonia (NH_3) (Mattila, 1998). NH_3 can also be oxidized and partly transformed to N_2O (Ferm, 1998).

In recent years, biochar has been evaluated as a tool for greenhouse gas suppression and carbon sequestration in soils (Lehmann, 2007). Biochar has shown the potential to suppress N_2O emissions in a wide variety of incubation studies (Bruun et al., 2011; Case et al., 2012; Spokas and Reicosky, 2009; van Zwieten et al., 2010b; Wang et al., 2011; Yanai et al., 2007) and in field studies using commercial fertilizers on soils used to grow rice (Liu et al., 2012b) or maize (Zhang et al., 2012). Biochar has also demonstrated the suppression of N_2O from pasture soil amended with bovine urine (Taghizadeh-Toosi et al., 2011). Effect of biochar on N_2O emissions in the above mentioned publications has largely been attributed to changes in soil aeration or moisture dynamics, an increase in pH, direct adsorption of N to char surfaces, microbial immobilization due to an increased C to N ratio, or microbial toxicity due to organic substances sorbed to char surfaces. However, other field studies reported no significant impact of biochar on N_2O emissions from soils amended with green manure on an organically managed 5-year crop rotation (Karhu et al., 2011) or pasture amended with chemical fertilizers (Scheer et al., 2011). In the above mentioned field experiments, biochar has also been demonstrated to decrease CH_4 emissions from soil potentially due to inhibition of methanogenic activity or lack of substrate availability (Liu et al., 2011), or to increase the potential of the soil as a CH_4 sink due to increased methanotrophy (Karhu et al., 2011; Zhang et al., 2012), though there was no demonstrated effect of biochar on CH_4 fluxes in Scheer et al. (2011). The potential for biochar to sorb NH_3 (Clough and Condron, 2010; Spokas et al., 2011b) has also been reported, though not in the field environment.

In addition to impacts on GHG emission, biochar has demonstrated potential to improve soil nutrient retention. Aged biochar may reduce soil total nitrogen loss through leaching (Lehmann et al., 2003) and may reduce loss of ammonium (NH_4^+) through leaching

through increased cation exchange capacity (CEC) (Ding et al., 2010; Singh et al., 2010). Fresh biochar has demonstrated the potential to decrease NO_3^- loss from soils amended with biosolids, possibly due to suppressed mineralization (Knowles et al., 2011) and to increase N use efficiency (Chan et al., 2007). Biochar has also demonstrated the ability to adsorb phosphate (PO_4^{3-}) (Lehmann, 2007) and reduce P in leachate (Laird et al., 2010; Novak et al., 2009; Yao et al., 2011). However, the presence of biochar has also contributed to an increase in total soil P and K^+ due to surface runoff (Schnell et al., 2012), and P and K^+ in leachate (Lehmann et al., 2003). All of the above nutrient-retention experiments were pot trials, soil-column experiments, or were described as lab- or greenhouse-based, with the exception of Knowles et al. (2011), which utilized undisturbed soil lysimeters that were removed from the field, but received natural rainfall outside an experimental station. None of the above experiments were field-based.

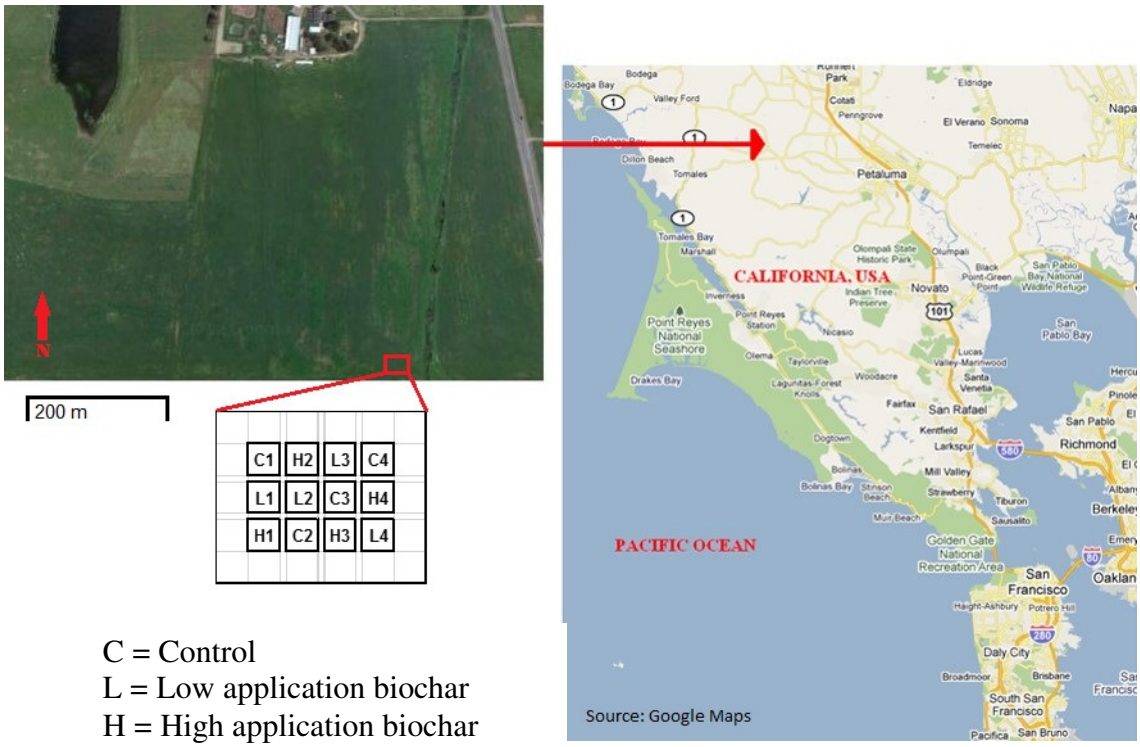
The purpose of the present study was therefore to: (i) examine the impact of biochar in a field-based study on N_2O, CH_4, and NH_3 emissions from a field used to grow ryegrasses for silage that has been co-amended with dairy manure, and (ii) examine the impact of biochar in a field-based study on nutrient leaching (NO_3^-, NH_4^+, PO_4^{3-}, and K^+) from a field used to grow ryegrasses for silage that has been co-amended with dairy manure.

5.2 Materials and methods

5.2.1 Site description and study design

The field study was conducted on a dairy farm in Petaluma, Sonoma County, California (Lat. 38º16' N; Long. 122º48'W) between 13 May 2011 and 9 May 2012 (Figure 1). This area has a mild Mediterranean climate where most of the precipitation falls as rain between October and April. Over the course of the experiment, there was approx. 692 mm of rainfall and average daily maximum and minimum air temperatures of 21.7º and 6.4º C, respectively (Figure 2). The site is located on a Blucher series fine sandy loam soil (thermic Fluvaquentic Haploxerolls) in a field used to grow annual ryegrass (*Lolium multiflorum*) with a topsoil bulk density (to 15 cm) of 0.8 g cm^{-3}, pH of 7.3, and C:N ratio of 10.1. Soil characteristics are presented in Table 1 and analytical methods are included in section 5.2.3. In a typical year, this field receives two to three applications of manure, but manure application only occurred once during the course of the experiment at a rate of approx. 150 m^3 ha^{-1} (410 kg N ha^{-1}) in late July 2011. Results of manure analysis are presented in Table 1. Harvesting of grasses occurred once in early June, once in early July, and once in late August. In order to facilitate biochar incorporation with minimal soil disturbance, an aerator with 7.6 cm coring tines (BlueBird, Charlotte, NC, USA) was used over the experimental area immediately following the second harvest and prior to biochar application. Disking occurred once in November, prior to the broadcasting of seeds. The site was temporarily flooded for a two-week period in late March 2012 following heavy rainfall. Data collection began approx. three weeks prior to the first harvest of 2011 and was concluded one day prior to the first harvest of 2012.

Figure 1: Aerial photograph and map of experiment site with graphics to indicate site location and arrangement of blocks within a randomized complete block design



C = Control
L = Low application biochar
H = High application biochar

Figure 2: Air temperature and rainfall data from 13 May 2011 to 9 May 2012 measured at the Santa Rosa Airport weather station in Santa Rosa, CA (approx. 20 km north of study site)

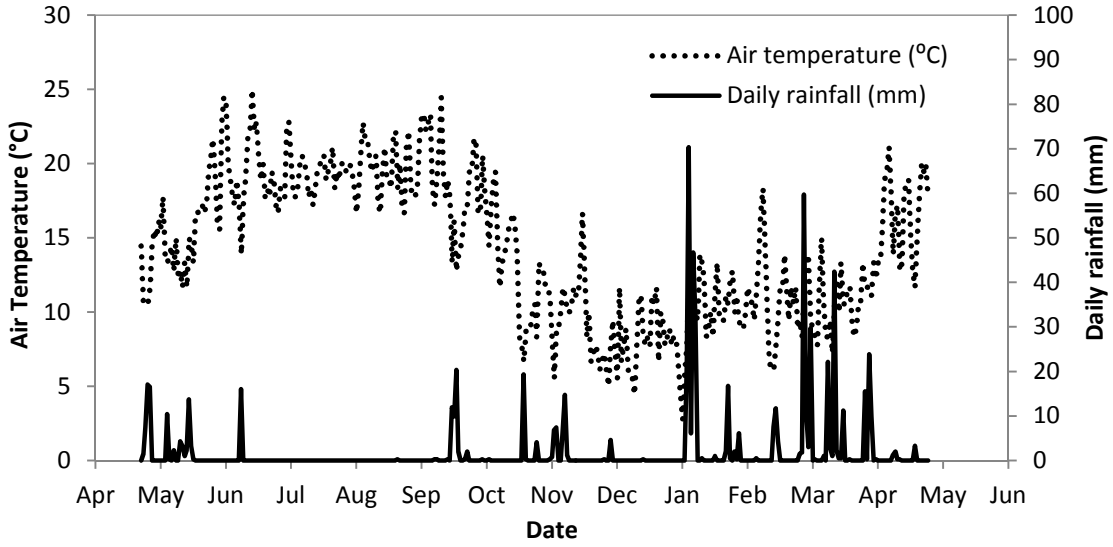


Table 1: Characteristics of the biochar, soil, and manure used in the experiment (methods described in Section 5.2.3)

Characteristics	Biochar	Soil	Manure
Dry Matter %	NA	NA	7.6
pH	7.9	7.3	NA
CEC meq/100g	3.2	30.1	NA
C mg g^{-1}	712	44.2	11.0[a]
Total N mg g^{-1}	9.1	4.4	2.7[a]
C/N ratio	78.2	10.1	4.1
Extractable PO_4^{3-}-P mg g^{-1}	0.82	200	0.03[a]
Extractable K^+ mg g^{-1}	7.2	1270	2.6[a]

NA = Not Analyzed

[a]Manure results are in g L^{-1}

The field site included 12 plots arranged together with a 5 m buffer zone, located 10 m from the southern edge of the field (a driveway) and 20 m from the eastern edge of the field (wild growth bordering a stream). The plots were arranged in four blocks with 1 m buffer zones between the blocks and individual plots. The field site contained three treatments: the control which received only manure, the low application biochar (LB) plots, which received manure and 5.7 t ha^{-1} biochar (thoroughly mixed into the manure as it was applied to the field) in late July, and the high application biochar (HB) plots, which received manure and 18.8 t ha^{-1} biochar in early July, immediately following second harvest and field aeration and 17 days prior to manure application. Biochar application rates were determined on a fresh weight basis, though moisture content of the biochar was presumed to be minimal. The treatments were organized in a randomized complete block design (RCBD) ($n = 4$). Each plot was 5 × 5 m and contained one collar for attaching a gas sampling chamber and one resin lysimeter (see section 5.2.4). A list of dates and sampling events is included as Table 2.

Biochar was obtained from New Earth Renewable Energy, Inc. (Seattle, WA, USA). This biochar was produced from waste pine (*Pinus*) material. The pine chips were heated to a

peak temperature of 550°C using a vacuum moving bed pyrolysis process. The biochar had an ash content of 17%, a pH of 7.9, a total surface area of 5.0 m^2 g^{-1}, and a total amount of 712 g C kg^{-1}, 9.1 g N kg^{-1}, 236 g O kg^{-1}, and 0.5 g S kg^{-1}. Further analysis is presented in Table 1 and analytical methodology is described in section 5.2.3.

Table 2 List of Events and Range of Soil Sampling Dates Included in Each Event

Event Name	Sampling Date Range
Biochar addition to HB plots	9 Jul 2011 - 16 Jul 2011
Slurry application (and biochar to LB)	28 Jul 2011 - 3 Aug 2011
Before harvest	11 Aug 2011 - 18 Aug 2011
Third harvest of 2011	22 Aug 2011 29 Aug 2011
Between harvest and first rain	8 Sep 2011 - 29 Sep 2011
First rain	4 Oct 2011 - 12 Oct 2011
Between rain and disking	16 Oct 2011 - 4 Nov 2011
Disking and seeding	12 Nov 2011 - 26 Nov 2011
Dry period following disking	2 Dec 2011 - 19 Jan 2012
Rain following dry period	23 Jan 2012 - 31 Jan 2012
Rain season before first harvest 2012	7 Feb 2012 - 9 May 2012

5.2.2 N_2O and CH_4 flux measurements

Measurements of trace GHG flux were made once per day for 7-10 days following each agricultural or meteorological event. These events included biochar application, manure application, harvest, disking/seeding, and rain following a dry period of greater than 30 days. Emissions were measured once per week between agricultural events. Gas samples were collected from vented-closed-flux chambers modeled after Hutchinson and Mosier (1981), which consist of a chamber lid that attaches to an in-site collar with additional rings to accommodate the height of grasses (when necessary). Collars were constructed of 20.3 cm diameter polyvinylchloride (PVC) rings with a height of 15 cm which were inserted into the ground to a depth of approx. 9-12 cm. Heights of the collars above the surface of the soil were measured immediately after collar placement and periodically thereafter to account for changes in headspace volume due to soil settling. Collars remained in the same position throughout and between sampling events and were only

removed when required for machine operations (harvest or disking), and were replaced as soon as possible afterwards. Chamber lids were constructed of PVC irrigation caps and covered with aluminum to reflect sunlight and mitigate temperature increases within the chamber during sampling events. From 14 February to 4 April 2012, an additional PVC ring with a height of 45.7 cm was inserted between the collar and chamber lid during sampling events, and from 11 April until 9 May 2012, two PVC rings (with a combined height of 91.4 cm) were inserted between the collar and chamber lid during sampling events, in order to accommodate the changing height of ryegrasses. PVC rings were held in place with rubber gaskets. A MagLev motor fan (Sunon, Inc., Kaohsiung, Taiwan, R.O.C.) and 9V battery were permanently affixed to the inside of the headspace lids prior to the 14 February sampling event in order to improve air circulation in the expanded chamber volume. Chamber lids and PVC rings were stored adjacent to collars between sampling events, at a distance to prevent obstruction of light or rainfall to the soil within the collar.

Prior to sampling, lids were affixed to the collars using a rubber gasket. Air samples were taken from the headspace via a rubber septum using an air-tight polypropylene syringe and hypodermic needle at 15, 30, and 45 minutes after closure of the headspace. Between 14 February and 9 May 2012, the fan was operated within the headspace for 1 min. prior to collection of each air sample. Two ambient samples were also taken from the field (at the same height above the soil as the height of the chambers at that time) at the beginning of each sampling event. Each 20 ml air sample was injected into a pre-evacuated 12 ml soda glass Exetainer vial (Labco Limited, Lampeter, UK). Glass vials were sealed with rubber septa and screw caps and pre-evacated using a vacuum pump. Pressurized samples were analyzed as soon as possible after collection (usually within 14 days) by gas chromatography.

Soil temperature at 15 cm depth was measured at the time of headspace closure, and air temperature was measured within each chamber at the time that each gas sample was collected using a thermocouple (Cole Palmer Instrument Co. Ltd., London, UK).

Gas analysis was performed using a Shimadzu GC-2014 Gas Chromatograph (Nakagyo-ku, Japan) equipped with an electron capture device (ECD) and a flame ionization detector (FID) with methanizer. The separation column was a HayeSep T capillary column (20.0 m x 0.32 mm i.d.) (Hayes Separations, Houston, TX, USA). Carrier gases were N_2 and He (95 kPa each). The ECD detector was set at 320°C, the FID was set at 250°C, and the oven temperature was 80°C. Retention times were 2.36 min for CH_4 and 5.67 min for N_2O, and the total time for one sample was set to 6.5 min. N_2O standards were 0.3, 0.9, 11, and (when appropriate) 100 ppm; CH_4 standards were 1.7, 9.8, and (when appropriate) 500 ppm. N_2O and CH_4 fluxes were tested for linearity (Hutchinson and Mosier, 1981) to determine the best flux and converted to $\mu g\ m^{-2}\ h^{-1}$ using the Ideal Gas Law. Cumulative emissions were calculated using the linear trapezoidal method.

Additionally, N_2O fluxes were normalized to daily average temperature by applying a Q_{10} function to the data. The Q_{10} coefficient was calculated according to the Arrhenius equation and applied to all of the data using the following equation (Parkin and Kaspar, 2003):

$$\text{Daily average gas flux} = R \times Q^{(DAT)/10} \quad (1)$$

Where R is the measured gas flux, DAT is the daily average air temperature and Q is the Q_{10} value. Fluxes that were equal to or less than 0 $\mu g\ m^{-2}\ h^{-1}$ were not included in the model and the Q_{10} coefficient was not applied to these data.

5.2.3 Soil sampling and chemical analysis of soil, biochar, and manure

Soil samples were collected three times within 7-10 days following each agricultural or meteorological event listed previously and once per week between events. At each soil sampling, 2-3 replicate soil samples were taken to a depth of 10 cm below surface vegetation, within 1 m of each chamber using a 2cm diameter auger and were bulked for analysis. Soil moisture content was determined gravimetrically by drying a subsample

for 24 hours at 105ºC. Water-filled pore space (WFPS) was calculated using measured bulk density and assuming a mineral particle size density of 2.65 g cm^{-3} (Robertson and Groffman, 2007). Soil pH was determined using 5 g of soil of soil that had been dried for 24 h at 60ºC, finely ground with a mortar and pestle, and sieved to 2mm. The soil was combined in a 1:1 ratio with deionized water, shaken for 30 min, and analyzed using a Seven Easy pH meter (Mettler Toledo International Inc., Columbus, OH, USA) with an Accumet combination pH glass electrode (Thermo Fisher Scientific, Waltham, MA, USA). The pH meter was calibrated using pH standards of 4, 7, and 10. For determination of ammonium (NH_4^+), nitrate (NO_3^-), total N, and dissolved organic carbon (DOC), a 0.5 M K_2SO_4 extract (50 ml) was prepared using 15 g of the bulked field-moist soil sample. NH_4^+ and NO_3^- were estimated colorimetrically (Doane and Horwath, 2003) using a spectrophotometer (Shimadzu UV-PharmaSpec 1700, Nakagyo-ku, Japan) which was calibrated using two blanks. NH_4^+ and NO_3^- concentrations were calculated from spectrophotometer data by linear regression using standards (0.1, 0.2, 0.5, 1, 2, and 3 ppm NH_4^+; 0.1, 0.5, 1, 5, and 10 ppm NO_3^-) and one sample from each dataset was analysed in triplicate as a quality control measure. Deionized water in 2:1 and 4:1 dilutions were used when concentrations exceeded the standards. Total N and DOC were ascertained sequentially by combustion, N determination by chemiluminescence and DOC determination using a non-dispersive infrared detector (NDIR) (Shimadzu TOC-V and TNM-1, Nakagyo-ku, Japan). Total N and DOC concentrations were determined by linear regression using standards (0, 5, and 100 ppm total N; 0, 25, and 100ppm DOC).

NH_4^+ and NO_3^- intensity values represent a measure of exposure of the soil microbial community to inorganic N for each event and were determined by calculating mean daily soil NH_4^+ and NO_3^- concentrations by linear interpolation between sampling events (Burton et al., 2008). For example, if NO_3^- concentrations in a soil sample taken from plot C1 on 9 July were 5.8 mg kg^{-1} and NO_3^- concentrations in a soil sample taken during the next sampling event two days later (11 July) from plot C1 were 8.3 mg kg^{-1}, the NO_3^- mean daily concentration for the period of time between those two sampling events would

be calculated as follows:

$$\frac{8.3\ \text{mg kg}^{-1} - 5.8\ \text{mg kg}^{-1}}{2\ \text{days}} = 1.25\ \text{mg kg}^{-1}\ \text{day}^{-1} \quad (2)$$

The mean daily concentration for this period of time would be 1.25 mg kg^{-1} day^{-1}. The annual or event NO_3^- intensity values are calculated as the summation of mean daily soil NO_3^- concentrations for the year or event.

Once every 28 days, air-dried soil samples (dried for > 2 weeks) were analyzed for phosphorus (P) and potassium (K^+). Exchangeable K^+ was extracted with 1 N ammonium acetate (pH 7) and analyzed by Inductively Coupled Plasma (ICP) (iCAP 6500 ICP Spectrometer, Thermo Fisher Scientific, Waltham, MA, USA). Available P was determined by the Olsen method (Olsen et al., 1954), based on an extraction of PO_4^{3-} from the soil by a 0.5 N sodium bicarbonate solution (pH 8.5) analyzed using an automated Flow Injection Analyzer (Lachat QuikChem 8500, Loveland, CO, USA). Reagent blanks were utilized in P and K^+ analysis, as well as duplicate samples for the first, last, and every tenth sample of each dataset.

Soils were analyzed for CEC and total C at the beginning and end of the experiment. CEC was determined by a barium replacement method using 1 N barium acetate in which a known quantity of Ca^{2+} was exchanged for barium and excess solution Ca^{2+} was measured. CEC was then determined by the difference in the quantity of the Ca^{2+} added and the amount found in the resulting solution. Blanks and 5, 50, and 100 mg L^{-1} Ca^{2+} standards were utilized, as well as duplicate samples for the first, last, and every tenth sample in each dataset. Analysis was conducted by ICP. Total C was determined using a Thermo Finnigan FlashEA 1112 Series dynamic flash combustion system (TCD) to combustion furnace coupled with a gas chromatographic (GC) separation system (Thermo Fisher Scientific, Waltham, MA, USA).

5.2.4 Resin lysimeters

Nutrient loss through leaching was assessed using *in situ* resin lysimeters (Kolberg et al., 1997; Paschold and Wienhold, 2008; Wienhold et al., 2009), which were interred in July, after biochar application to HB plots and prior to manure application. A metal cylinder (4.75 cm i.d.) was inserted vertically 17 cm into the soil and immediately removed with an intact soil core. 2 cm of soil were removed from the bottom of the cylinder and replaced by a nylon bag containing 10 g of a 1:2 mixture of Na-saturated cation (C-249) and Cl-saturated anion (ASB-1P) resin (IONAC resins, LANXESS Sybron Chemicals, Birmingham, NJ, USA). The bottom of the lysimeter was covered by a heavy nylon cloth held in place by a metal coupling. The lysimeter was then reinserted into the original hole. Once every 28 days, the lysimeters were removed from the ground and the resin bag was replaced. Resin bags were subjected to a serial extraction, similar to that of Kolberg et al. (1997). Resin bags were immersed in five separate volumes (25 ml each) of 2M NaCl and each shaken for 15 min before combining extracts for analysis.

Exchangeable K^+ in the extracts was determined by ICP. PO_4^{3-} and NH_4^+ concentrations were determined spectrophotometrically using an automated Flow Injection Analyzer (Lachat QuikChem 8500, Loveland, CO, USA). Reagent blanks were utilized as well as duplicate samples for the first, last, and every tenth sample of each dataset. NO_3^- was estimated colorimetrically using a spectrophotometer (Shimadzu, UV-PharmaSpec 1700, Nakagyo-ku, Japan). Reagent blanks were used and one sample was analyzed in triplicate from each dataset. Cumulative nutrient loss were determined by calculating the sum of leached amounts (expressed as kg ha^{-1}) from the duration of the experiment.

5.2.5 Ammonia

NH_3 emissions were determined once during the study period and were estimated using a method similar to that used by Matsushima et al. (2009). Immediately following manure application, an open sample cup containing 20 ml of 0.25 M H_2SO_4 was positioned within each experimental plot and covered with a 2.5 L high density polyethylene container (Phenix Research Products, Candler, NC, USA). After 10 days, the traps were collected.

The amount of volatilized NH_3 from the soils was determined through titration of 10 ml of solution from each trap. Prior to titration, 1-2 drops of phenolphthalein were added to the solution. 0.5 M NaOH was slowly added using a burette until a color change was observed. Based on the amount of NaOH added to the H_2SO_4 trap, the amount of $(NH_4)^2SO_4$ present in the trap was calculated and NH_3-N emission was determined. All titrations were conducted in duplicate and a mean value of results was used in calculations.

5.2.6 Plant tissue

At the end of the experiment, 0.25 m^2 within each plot was harvested by hand at the soil level and grasses dried for 24 h at 60ºC to determine yield. Plant P and K^+ were determined using a nitric acid/hydrogen peroxide microwave digestion and determined using ICP. Plant N was determined by combustion coupled with thermal conductivity/IR detection (LECO TruSpec CN Analyzer, St. Joseph, MI, USA). Reagent blanks were utilized, as well as duplicate samples for the first, last, and every tenth sample of each dataset.

5.2.7 Statistical analysis

Treatment effects on cumulative N_2O, cumulative CH_4, NH_3, total carbon, NO_3^- intensity, NH_4^+ intensity, CEC, grass yield, plant nutrient results, and cumulative resin nutrient results were assessed using a one-way analysis of variance (ANOVA) with treatment type included as a fixed effect. Additionally, the interaction of treatment effects and event on cumulative N_2O, cumulative CH_4, NO_3^- intensity, and NH_4^+ intensity were also assessed using a two-way ANOVA with treatment type and event name included as fixed effects. A list of event names and the range of sampling dates included in each event are included in Table 2. Soil pH and available P and K^+ concentrations were assessed using a two-way ANOVA with treatment type and day number included as fixed effects and replicates considered random effects. For all ANOVA models, block number was included as a random effect. pH values were log-transformed (natural log) prior to statistical analysis; all other parameters were square root transformed prior to statistical analysis in order to

achieve normality. All transformed datasets were normally distributed unless otherwise noted below. Post-hoc comparisons were performed using Tukey's correction for multiple testing. Model fit was assessed using graphical analysis of residuals, a Shapiro-Wilk test for normality, and the O'Brien test for equal variance. The 0.05 confidence interval was used to determine statistical significance. Pearson correlation coefficients were calculated between resin data and N_2O fluxes and environmental parameters such as rainfall. All statistical analyses were conducted using JMP 9.0.0 (SAS Institute, Cary, NC, USA).

Monthly cumulative NO_3^-, NH_4^+, K^+, and P were analyzed using monthly cumulative rainfall, treatment, and the interaction between rainfall and treatment as fixed effects, and plot as a clustering variable. The monthly cumulative value was the total amount that accrued during the interval between one observation and the next. As the repeated measures design was likely to cause correlation between observations on the same plot and as the form of this effect was unknown, a Generalized Estimating Equation (GEE) (which is particularly robust to misspecified correlation) was used to fit the model. These analyses were conducted using R (R Development Core Team, 2012) and the geepack library (Højsgaard et al., 2006).

5.2.8 Boundary line approach

A boundary line approach was used to establish the relationship between N_2O fluxes and soil or air temperature, as well as Q_{10} corrected N_2O fluxes and WFPS (Schmidt et al., 2000; Lee et al., 2008) The boundary line represents optimum N_2O flux and was fitted to the points that were the 99% percentiles of all each of eight equidistant sections of soil temperature or WFPS.

5.3 Results and Discussion

5.3.1 Soils and plant data

There was no significant difference between treatments for yield or nutrient concentrations in plant tissue (Table 3). This is consistent with some studies (Gaskin et al., 2010; Schnell et al., 2012) but not in others that have reported an increase in plant growth from soils amended with biochar and fertilizer (Lehmann et al., 2003; Sinclair et al., 2009; Steiner et al., 2007; Van Zweiten et al., 2010b). The majority of the studies that have reported increased plant growth or plant uptake of nutrients have used highly weathered Ferrosols with pH values below 5.0 (Lehmann et al., 2003; Sinclair et al., 2009; Steiner et al., 2007; Van Zweiten et al., 2010b), though Van Zweiten (2010b) also reported enhanced growth of soybeans and radishes from a Calcarosol with a pH of 7.7 that had been amended with biochar. Overall, it is likely that the effect of biochar on plant growth and nutrient uptake would be more pronounced in soils with low nutrient contents or a low pH, unlike the soils in the present experiment.

Soil samples taken in May 2012 indicated that soils in HB plots contained significantly higher total carbon content than the other treatments ($p = 0.0253$), though these data failed the equal variance test. These data indicate some recalcitrance to microbial mineralization of biochar C, which has been reported by others (Liang et al., 2006; Novak et al., 2010). Soil pH results also indicated a treatment effect ($p = 0.0456$), and although average pH of soils from the HB plots was higher than average pH of soils from LB plots or the control, Tukey test results indicated no significant difference between mean values. An increase in soil pH from biochar amended soils has been reported in other studies (Streubel et al., 2011; van Zwieten et al., 2010a; van Zwieten et al., 2010b). There was no significant treatment effect for soil CEC ($p = 0.5784$), soil available P ($p = 0.8860$), or soil available K^+ ($p = 0.4489$).

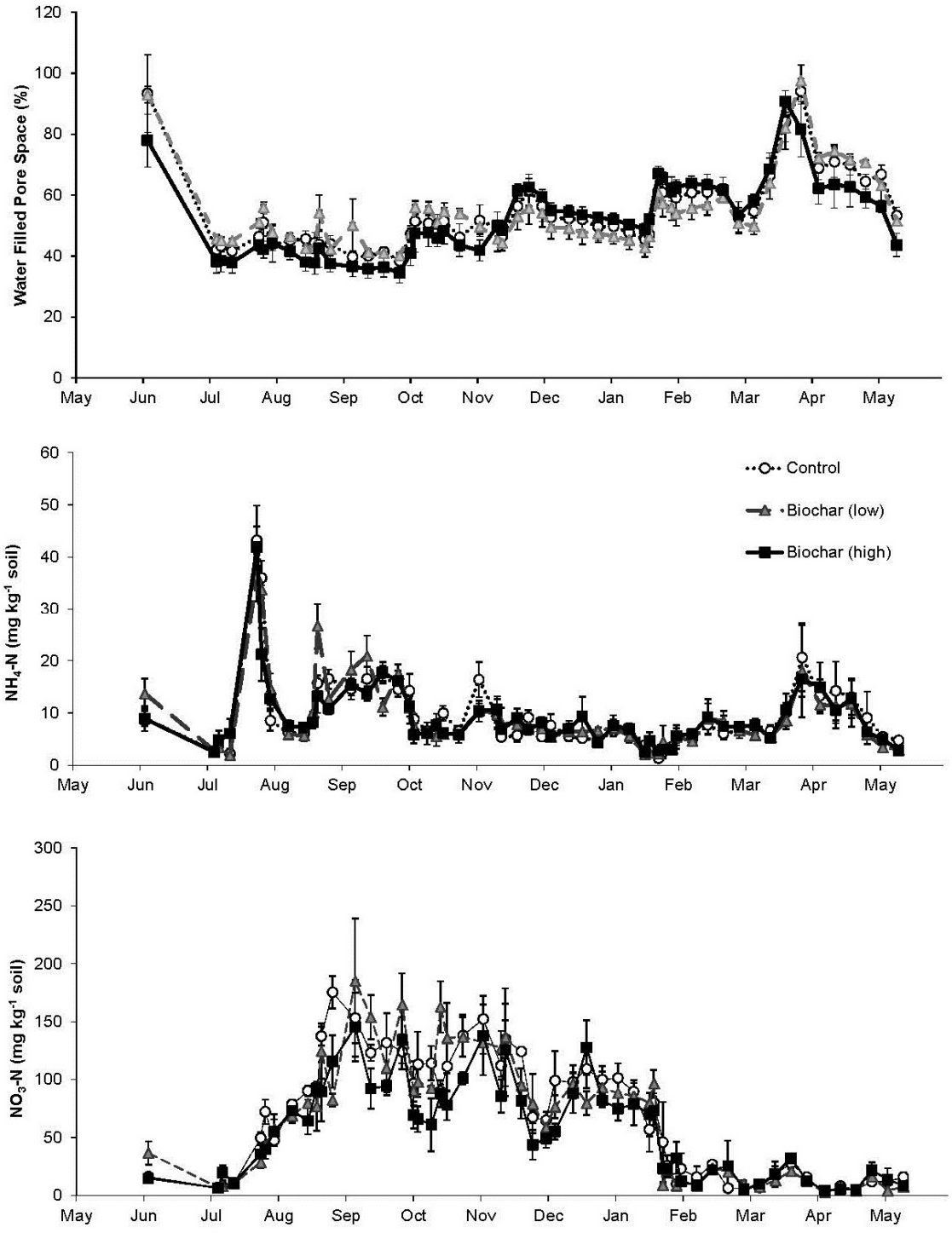
Table 3 Yield, Plant Tissue, and Soils Data (End of Experiment), means and standard errors ($n = 4$)

	Control	Biochar (Low Application)	Biochar (High Application)
Yield kg ha^{-1}	8840 (742)a	10,100 (1690)a	9340 (1640)a
Plant Data:			
Total N mg g^{-1}	20.2 (0.6)a	19.9 (1.8)a	17.6 (1.9)a
Total P mg g^{-1}	3.50 (0.10)a	3.32 (0.33)a	3.11 (0.18)a
Total K mg g^{-1}	36.7 (2.0)a	35.6 (4.1)a	35.4 (2.8)a
Soils Data:			
Total N mg g^{-1}	5.71 (0.06)a	5.72 (0.20)a	6.20 (0.55)a
Total C mg g^{-1}	59.5 (4.7)b	61.4 (3.5)b	104 (18.1)a
Extractable PO_4^{3-}-P mg g^{-1}	173 (3)a	161 (6)a	172 (9)a
Extractable K^+ mg g^{-1}	1220 (16)a	1220 (33)a	1230 (107)a
CEC meq/100 g	29.7 (0.5)a	29.9 (0.7)a	29.0 (0.6)a

Different lowercase letters indicate significant differences at $p < 0.05$.
Values in parentheses are standard errors of the mean.

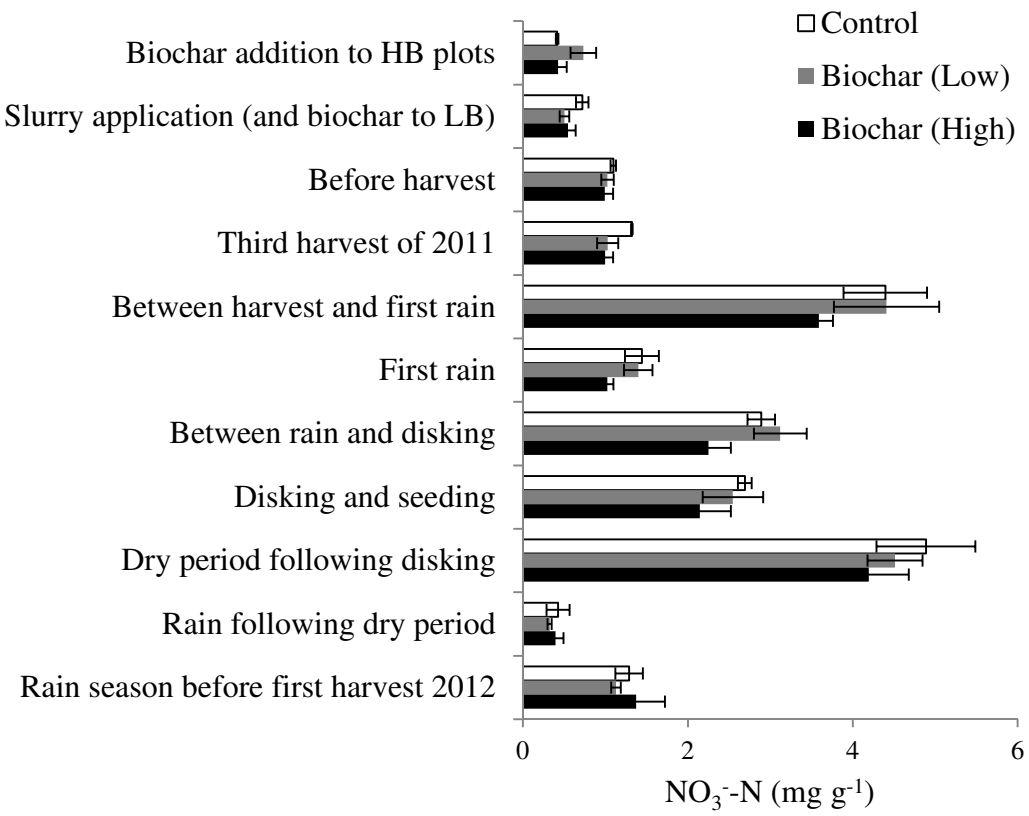
Available NH_4^+ in grassland grassland soils is expected to be consistent at 3-9 µg NH_4^+-N g^{-1} throughout the year (Richardson, 1938). Soils in the present study were in this range through much of the year, but peaked far above this range (though within the range reported by Chadwick et al., 2000) for a brief time immediately following manure addition, for approx. 40 days following the third harvest of grasses (until the first rain), and again during the period that the soil was saturated (Figure 3). An increase in N mineralization following harvest has been documented in other studies (Rimski-Korsakov et al., 2012), and N mineralization that has been enhanced by increased soil moisture content has also been reported (Chen et al., 2012).

Figure 3: Water-filled pore space (WFPS) and mineral N contents of soil, means and standard errors (n = 4)



There was no significant difference between treatments for annual cumulative NO_3^--N intensity or cumulative NH_4^+-N intensity (p = 0.1046 and 0.6363, respectively), and no significant treatment effect on NH_4^+-N intensity when analyzed by event (p = 0.7452). However, when NO_3^--N intensity was analyzed by event with treatment and event as fixed effects, there was a significant treatment effect (p = 0.0008) with HB plots exhibiting a significantly lower soil NO_3^--N intensity value than the other treatments. Additionally, there was not a significant interaction effect (p = 0.5052), indicating that the extractable NO_3^- concentration of the HB soils was consistently lower than the other treatments throughout most of the year (Figure 4). This suggests that the presence of biochar may have had a significant and lasting effect on the nitrification process in these plots. The impact of biochar on the nitrification process has been demonstrated in some studies (Clough et al., 2010; Deenik et al., 2010), though other studies have inferred no nitrification inhibition (Bruun et al., 2011; Singh et al., 2010), or have even reported an increase in nitrification rates due to presence of biochar (van Zwieten et al., 2010b). It has been demonstrated that an increase in P and/or K^+ addition to soil can decrease the amount of NO_3^- in soil (Yang et al., 2012), thus it is also possible that the P and K^+ content in the char may have impacted the soil NO_3^- content in the HB plots. However, given that there was no significant effect of biochar on soil P and K^+ concentration and given the low P concentration of the char and the high solubility of K^+, this would have been likely to be a short term effect that would not have lasted long past the first rain event. It is possible that the impact on nitrification rates is due to hydrocarbons sorbed to biochar having an initial effect of suppression of soil microbial communities (Spokas et al., 2009; Spokas, 2010; Spokas et al., 2011a).

Figure 4: NO_3^- intensity by event, means and standard errors (n = 4)



5.3.2 Gaseous emissions

Effect of biochar

Although the lowest average cumulative N_2O, CH_4, and NH_3 emissions were from HB plots (Table 4), there was no significant difference between treatments (p = 0.1517, 0.4957, and 0.2850, respectively), which was similar to GHG results presented in Scheer et al. (2011). Additionally, there was no significant difference between treatments when N_2O and CH_4 emissions were analyzed by event (p = 0.7393 and 0.7716, respectively). Spatial variability may have contributed to the absence of a significant treatment effect, as has been reported in other field-based studies of gaseous emissions (Chadwick et al., 2000; Fangueiro et al., 2008; Wulf et al., 2002). If continued over several seasons, biochar may have demonstrated a significant effect on cumulative emissions over time

either due to effects of the aging of the biochar in the soil or due to the decrease of standard error following acquisition of more results. Additionally, the treatment effect on NO_3^- intensity values may eventually be reflected in significantly lower average N_2O emissions from HB plots over the long term. Although biochar has demonstrated the potential to inhibit N_2O production, this has often occurred under laboratory conditions using biochar at high application rates up to 150 t ha^{-1} (Yanai et al., 2007) or 240 t ha^{-1} (Spokas et al., 2009), which would not have been practical in a field setting. Additionally, biochar can have a diverse effect on N_2O emission based on the type of char that is used and the soil to which it has been applied (Spokas and Reicosky, 2009), and it is possible that a different type of char may have had a more pronounced effect in this setting.

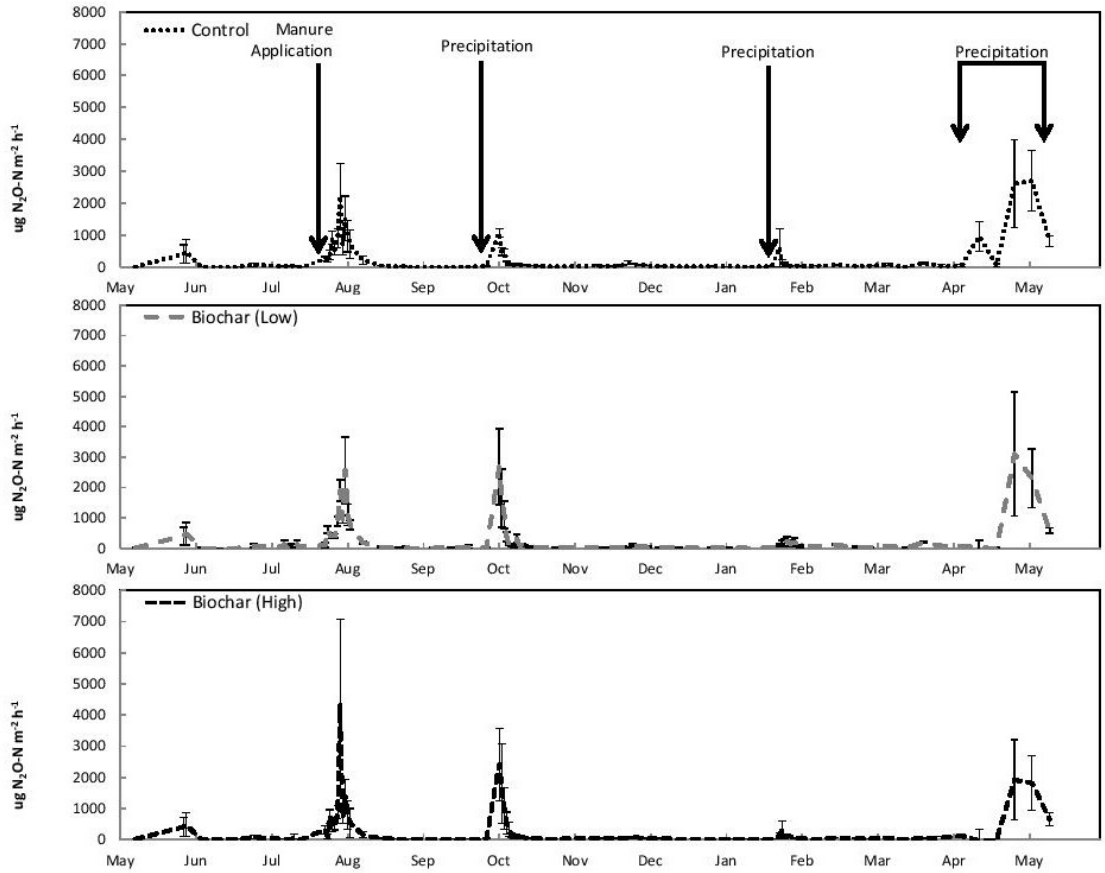
Table 4 Net total gaseous emissions, means and standard errors (n = 4)

Treatment	CH_4-C	EF[a]	N_2O-N	EF	NH_3-N[b]
	g m^{-2} yr^{-1}	*%*	*g m^{-2} yr^{-1}*	*%*	*mg m^{-2}*
Control	0.88 (0.64)a	0.5 (0.3)	1.9 (0.3)a	4.7 (0.7)	1.47 (0.29)a
Biochar (low application)	0.98 (0.42)a	0.6 (0.2)	2.0 (0.5)a	4.9 (1.3)	1.41 (0.22)a
Biochar (high application)	0.54 (0.33)a	0.3 (0.2)	1.6 (0.4)a	4.0 (1.0)	0.96 (0.08)a

Different lowercase letters indicate significant differences at $p < 0.05$
[a]Emission Factor (% of total C or N applied as manure)
[b]NH_3-N was measured one time for a 10 day period following manure application

Suppression of CH_4 emission from other studies been attributed to increased aeration (van Zwieten et al., 2009) due to macropores in wood-derived char (Downie et al., 2009), however there was no significant difference in soil bulk density values between treatments. Karhu et al. (2011) reported an increase in CH_4 uptake from biochar-amended soils, however these soils exhibited a net uptake from all treatments over the course of the experiment, unlike the present study.

Figure 5: N_2O emissions from the control, low biochar application treatment, and high biochar application treatment; means and standard errors (n = 4)



N_2O Emissions

N_2O emissions varied significantly throughout the year, the highest peaks occurring in the two weeks following manure application, the five days following a rain event (after a dry spell of greater than 30 days), and in the five weeks following prolonged soil saturation (and temporary flooding) caused by frequent and heavy rains in March (Figure 5). N_2O peaks are highly episodic (Scheer et al., 2011), with the highest peaks typically following N application or rainfall events (Fangueiro et al., 2008; Hyde et al., 2006; Merino et al., 2001; Phillips et al., 2007; Syväsalo et al., 2006; Wulf et al., 2002). The highest mean rate of emission during the study was 4311 µg N_2O-N m^{-2} h^{-1}, which was within the range of other experiments measuring N_2O emissions from surface applied

manure (Jones et al., 2007; Rees et al., 2004), but higher than others (Chadwick et al., 2000; Sherlock et al., 2002), which had peak N_2O emission rates that were approx. one-quarter and one-third of the present study, following total N application rates of 270 kg N ha^{-1} and 366 kg N ha^{-1}, respectively. The manure applied in Chadwick et al. (2000) was applied in three events over the course of the year rather than one event, which would have resulted in lower soil NH_4^+ concentrations prior to the time of peak emissions than the present experiment, thereby limiting the amount of N available to the nitrifying and denitrifying bacteria limiting the resulting N_2O emission. Although Sherlock et al. (2002) made a single manure application, the soil NH_4^+ concentration at the time of this event was one-quarter that in the present study. The NH_4^+ in the present study was likely to have been quickly nitrified following the addition of manure C and moisture, resulting in higher peak N_2O emissions. The intensity of N_2O emission following manure application is dependent on the increase of soil N and C availability, which varies depending on the characteristics of the soil and manure, and the manure application rate (Velthof et al., 2003). As evidenced in the soil NH_4^+ data (Figure 3), these soils demonstrated soil mineral N at concentrations higher than average for grassland systems, which (in conditions favoring nitrification and denitrification processes) would be likely to emit higher than average amounts of N_2O.

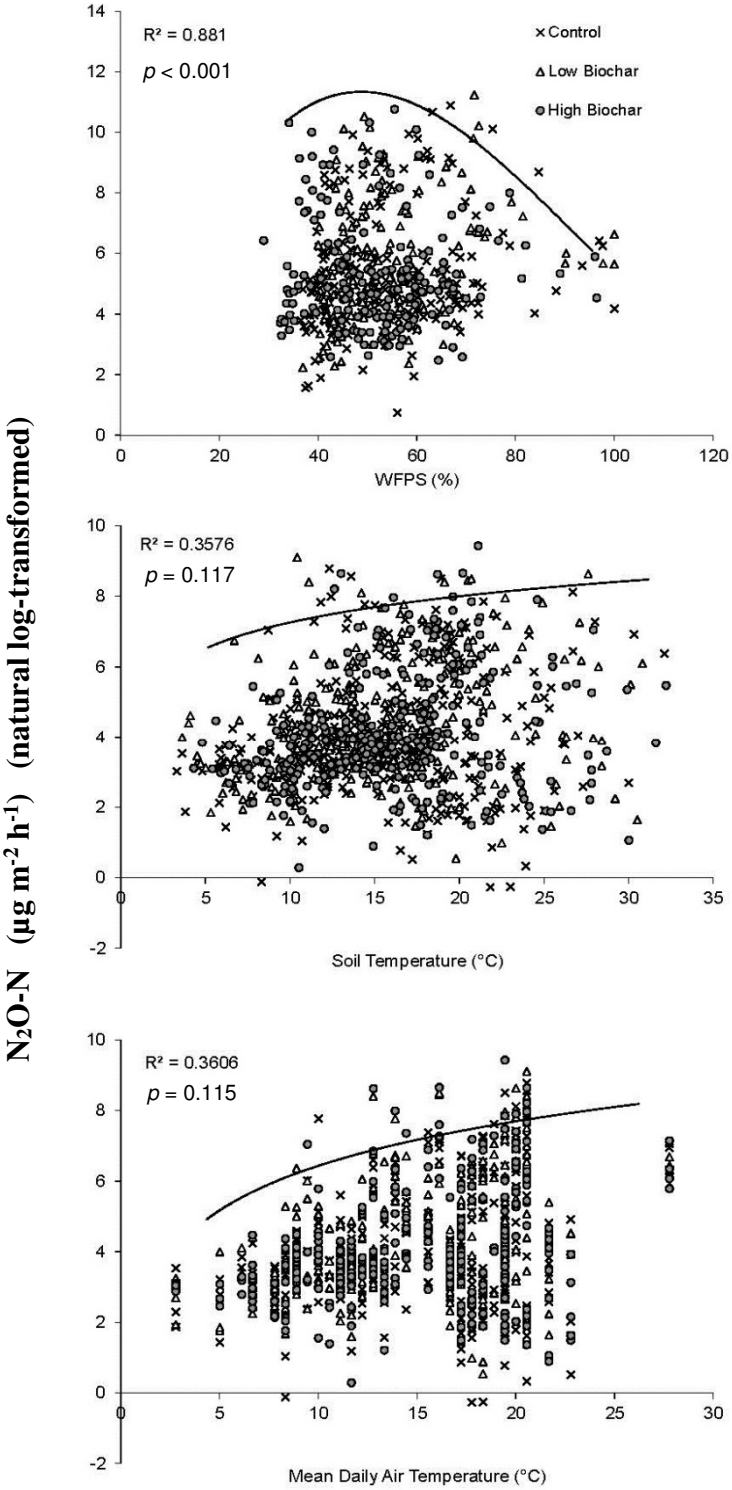
Average cumulative emissions ranged from 1.6 to 2.0 g N_2O-N m^{-2} y^{-1} which accounted for approx. 4.0-4.9% of applied manure N (Table 4). These cumulative emissions and emission factors were higher than other field experiments measuring emission from surface-applied manure (Jones et al., 2007; Sherlock et al., 2002; Syväsalo et al., 2006), often an order of magnitude or more (Chadwick et al., 2000; Fangueiro et al., 2008; Velthof and Mosquera, 2011), though within the IPCC default emission factor range (IPCC, 2001). This was in part due to the high N application rate, which can increase emission factors (Velthof and Mosquera, 2011). As emission rates from this study were within the range of other studies, these emission factors also indicate that peak emissions occurred more frequently and/or for longer periods of time than other studies. The extended N_2O peak flux period five weeks following heavy rain and flooding in March

2012 accounted for approx. 59%, 48%, and 42% of the annual cumulative emission from the control, LB, and HB treatments, respectively. These conditions were distinct from those in other studies, contributing to higher cumulative emissions and emission factors.

Boundary Line Approach

Based on the Arrhenius equation, a Q_{10} value of 2.80 was applied to the N_2O data. Regression analysis of N_2O results with soil temperature and average daily air temperature, and Q_{10} corrected N_2O results with WFPS indicated that there was no significant relationship ($r^2 = 0.016$, 0.04, and 0.003, respectively; $p < 0.0001$, $p < 0.0001$, and $p = 0.1652$, respectively). The scattergram of N_2O flux and WFPS reflect that soils never fell below 28.9% WFPS and indicate that N_2O emissions peaked around 45-55% WFPS (Figure 6), lower than the 55-60% suggested by Doran et al., (1988). In the present study, the highest emissions occurred between 34 and 75% WFPS and were limited in extremely wet conditions (>75% WFPS). This is similar to emission patterns described by others (Dobbie and Smith, 2001). Soil moisture controls the level of microbiological activity and oxygen availability, and these results indicate that pulse emissions were primarily the result of enhanced denitrification activity. N_2O emissions also increased with increasing temperature. Highest emissions occurred between air temperatures of 9 and 20°C, and with soil temperatures between 10 and 27°C. The period of highest (and most prolonged) N_2O emission occurred over a five week period beginning in late March, following a period of soil saturation (at which time there was a peak in soil N mineralization), when the soils were consistently at an optimal WFPS (Figure 3) and mean daily air temperature was above 10.5°C (Figure 2). These emissions were likely due to a synergistic effect of sufficient soil available N, consistent heavy rainfall leading to higher WFPS values, and the rising temperatures in the spring.

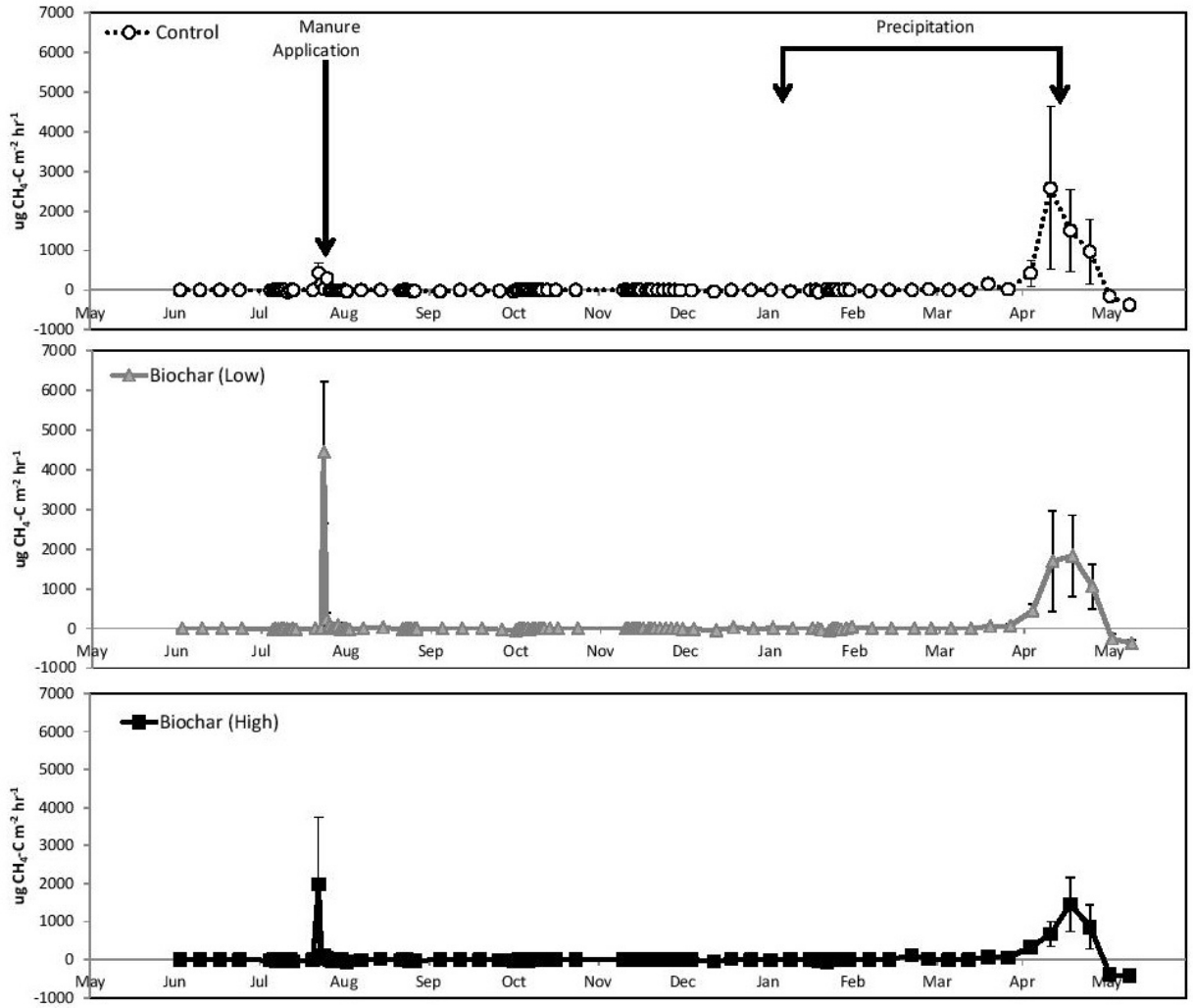
Figure 6: Scattergrams and boundary lines for N_2O flux plotted against water filled pore space, soil temperature, and mean daily air temperature



CH_4 Emissions

CH_4 emissions peaked immediately following application of manure, but returned to background levels within 5 days following application, which is similar to the pattern of emission reported by others (Chadwick and Pain, 1997; Chadwick et al., 2000; Flessa and Beese, 2000; Rodhe et al., 2006). This is due to release of dissolved CH_4 produced in the manure during storage and prior to application and then due to the metabolism by methanogens of volatile fatty acids in the manure-amended soil (Sherlock et al., 2002; Wulf et al., 2002). CH_4 emissions peaked again in the two weeks of soil saturation due to heavy rainfall in late March and the four weeks that followed this event, and was followed by a two-week period of high net soil CH_4 oxidation, in which average rates of CH_4 consumption for all treatments were greater than 370 µg CH_4-C m^2 h^{-1} (Figure 7). Peak rates following manure application were approx. 4450 µg CH_4-C m^{-2} h^{-1}, which were lower than those reported by Chadwick et al. (2000) and Fangueiro et al. (2008), but higher than those reported in an earlier study by Chadwick and Pain (1997) and Sherlock et al. (2002), in which case there were much lower manure application rates than in the present study. The CH_4 peak following soil saturation has not been demonstrated in similar grassland experiments as flooding is not typical for grassland agriculture, but similar peaks have been demonstrated in manure-amended rice fields following intentional flooding and irrigation due to methanogenisis following O_2 depletion (Qin et al., 2010; Liu et al., 2012a). The subsequent period of CH_4 consumption followed as O_2 supply increased, with the high water content of the soil providing an optimal state for CH_4 uptake (Scheer et al., 2011). CH_4 consumption may have also been affected by the induction of methanotrophic capacity through elevated CH_4 (Bender and Conrad, 1995) or NH_4^+ concentrations (De Visscher and Van Cleemput 2003) following the flooding. Additionally, the loss of mineral N via leaching and denitrification following the heavy rain and flooding may have enhanced CH_4 oxidation, as mineral N has been demonstrated to have an inhibitory effect on CH_4 oxidation in soils (Dubey et al., 2002). This is evidenced in the present experiment by low soil mineral N concentrations during this time period (Figure 3), as well as increased N_2O emission and NH_4^+ leaching (Figures 5, 8).

Figure 7: CH_4 emissions from the control, low biochar application treatment, and high biochar application treatment; means and standard errors (n = 4)



Average cumulative emissions ranged from 0.54 to 0.98 g CH_4-C m^{-2} y^{-1}, or 0.3% and 0.6% of total applied manure C, respectively (Table 4). As with N_2O results, CH_4 cumulative emissions were higher than those of other field-based experiments assessing GHG emissions from manure-amended soil. Indeed, Syväsalo et al. (2006) reported a net annual uptake of CH_4. However, the cumulative results prior to the peak following soil saturation were within the range of other studies (Chadwick and Pain, 1997; Fangueiro et

al., 2008), and cumulative emissions for this study were well below those reported for manure amended rice fields (Qin et al., 2010; Liu et al., 2012a).

NH_3 Emissions

Average NH_3-N emissions in the 10 days following application of manure to soil ranged from 0.98 to 1.47 mg NH_3-N m^{-2} (Table 4). This was higher than those described by Fangueiro et al. (2008) (who utilized a closed chamber technique), but orders of magnitude lower than other experiments which utilized a through flow system, the equilibrium concentration method, or the mass balance micrometeorological method (Amon et al., 2001; Rodhe et al., 2006; Sherlock et al., 2002). It has been demonstrated that closed systems can introduce artifacts that result in suppression of NH_3 emissions (Sherlock et al., 2002). However, comparisons between treatments are valid as the same system was used for all treatments.

5.3.3 Resin lysimeters

Cumulative loss of PO_4^{3-}-P and K^+ were approx. 3.7-5.5 kg ha^{-1} y^{-1} and 169-257 kg ha^{-1} y^{-1}, respectively. Cumulative loss of NH_4^+-N and NO_3^--N were approx. 3.0-4.1 kg ha^{-1} and 89-119 kg ha^{-1}, respectively (Table 5). PO_4^{3-}-P and K^+ extracted from resins increased in months of heavy rainfall and decreased markedly in months of low rainfall. The correlation coefficients for cumulative rainfall (within each period of time in which the resins were interred) with P and K^+ extracted from resins were each approx. 0.3 ($p < 0.0001$ for both). Correlation coefficients for amount of rainfall with NH_4^+-N and NO_3^--N extracted from resins were both less than 0.1 ($p = 0.1574$ and $p = 0.001$, respectively). This indicates that rainfall was a dominant factor in P and K^+ release from soils, and that other factors (such as soil microbial activity) were more important with respect to the release of mineral N. Highest average NH_4^+-N loss was during the first three months of resin use (August through October, encompassing the first rainfall). NO_3^--N extracted from resins was highest in the three months (October to December) following the first rainfall, and again in February 2012, immediately after a rainfall which had followed a long dry spell (Figure 8).

Table 5 Cumulative nutrients adsorbed by resins, means and standard errors (n = 4)

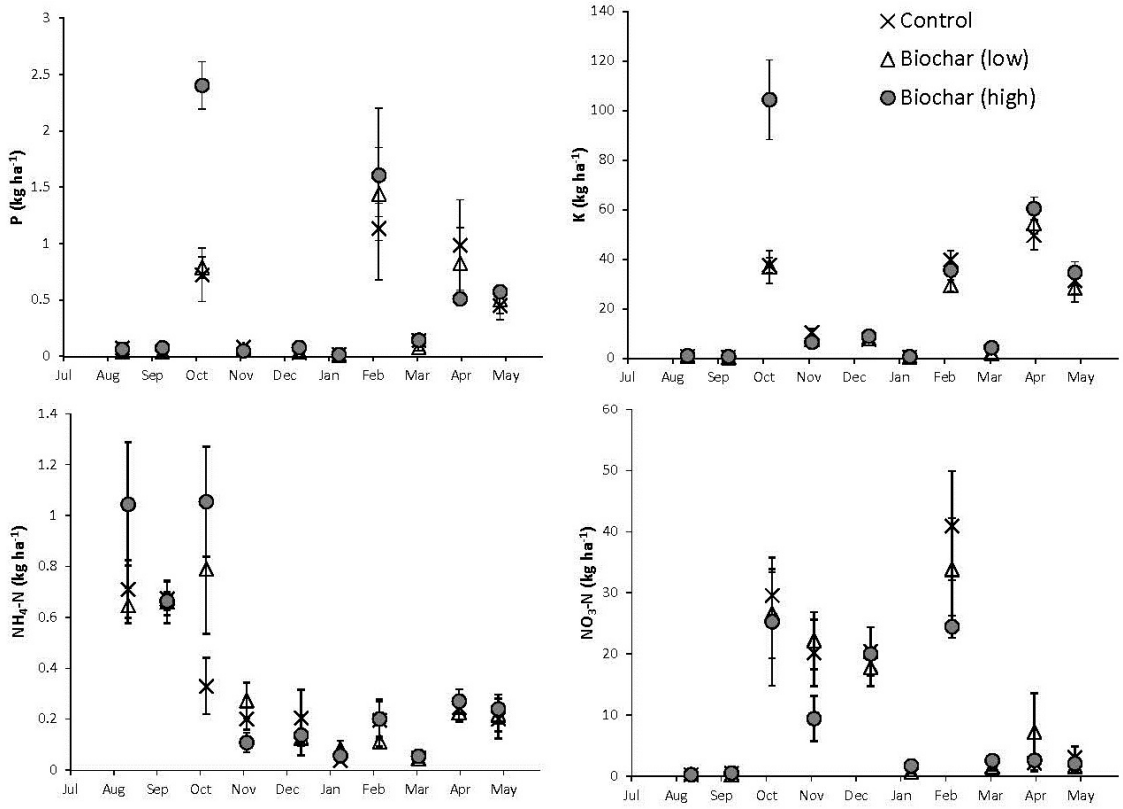
Treatment	NH_4^+-N		NO_3^--N		PO_4^{3-}-P		K^+	
	kg ha $^{-1}$ y $^{-1}$	*%* [a]	*kg ha $^{-1}$ y $^{-1}$*	*%* [a]	*kg ha $^{-1}$ y $^{-1}$*	*%* [b]	*kg ha $^{-1}$ y $^{-1}$*	*%* [b]
Control	3.0 (0.3)a	0.7 (0.1)	119 (5)a	29 (2)	3.7 (0.7)a	72 (11)a	181 (15)b	47 (4)a
Biochar (Low Application)	3.2 (0.4)a	0.8 (0.1)	112 (17)a	27 (4)	3.8 (1.2)a	39 (13)ab	169 (8)b	39 (2)a
Biochar (High Application)	4.1 (0.5)a	1.0 (0.1)	89 (7)a	22 (2)	5.5 (0.1)a	27 (1)b	257 (28)a	49 (5)a

Different lowercase letters indicate signficant differences at $p < 0.05$

[a] % of total N applied as manure

[b] % of total P or K applied as manure and/or biochar

Figure 8: PO_4^{3-}-P, K^+, NH_4^+-N and NO_3^--N retrieved from resin extracts, means and standard errors ($n = 4$)



There was no significant difference between treatments for cumulative NO_3^--N extracted from resins ($p = 0.2950$), though NO_3^--N loss from HB plots was significantly lower than the control in February, after a high-rainfall period following a long dry spell ($p = 0.0361$). Peaks in resin NO_3^- for all treatments occurred during heavy rains following a dry period, in which the soil microbial communities had sufficient time to mineralize and nitrify manure N. Resins collected after the first rain event following biochar and manure application indicated a significant treatment effect for NH_4^+-N ($p = 0.0002$), with the HB plots demonstrating significantly higher NH_4^+ release than the control plots. There was no significant difference in NH_4^+ concentrations between treatments in any other set of resin extracts, but cumulative NH_4^+-N results indicated a weak treatment effect (p =

0.0610). This suggests that the presence of biochar in the biochar-amended plots may have enhanced mineralization or inhibited nitrification prior to the first rain event, but that the soil was not able to retain the NH_4^+. Enhancement of mineralization of soil N by biochar has been demonstrated in some studies (Yoo and Kang, 2012) though decrease in N mineralization has been demonstrated in others (Dempster et al., 2012).

Resins collected after the first rain event following biochar and manure application indicated a significant treatment effect for PO_4^{3-}-P ($p < 0.0001$) and K^+ ($p < 0.0001$), with the HB plots demonstrating significantly higher PO_4^{3-}-P and K^+ values than the LB and control plots. This was likely due to loss of P and K^+ in the water soluble ash content that was rinsed from the char with the first rain event. There was significantly higher PO_4^{3-}-P released from HP plots in early February ($p = 0.0430$), during a high rainfall following a dry period, possibly due to release of P from decomposing biochar (Unger and Killorn, 2011). At this same time, there was a decrease in K^+ loss from LB plots ($p = 0.0065$) possibly indicating a short term suppression of loss of manure K^+, as was reported following a mass balance analysis conducted by Laird et al. (2010). The cumulative K^+ results demonstrated a significant increase in K^+ leaching from HB plots over that of the control ($p = 0.0176$), however the cumulative PO_4^{3-}-P results indicated no treatment effect ($p = 0.2098$). Conversely, there was no significant difference between treatments for resin K^+ expressed as a percentage of K^+ applied as manure and biochar ($p = 0.1922$), but there was a significant difference between treatments for resin PO_4^{3-}-P expressed as a percentage of P applied as manure and biochar ($p = 0.0259$), with HB plots demonstrating significantly lower values than control plots, indicating either that a lower percentage of P was in extractable form given the P within the biochar that has been made unavailable via pyrolysis (Chapter 2), or that the presence of biochar induced a higher percentage of manure P to be converted to organic P.

Increases in K^+ leaching from soil following biochar application have been reported by Lehmann et al. (2003), however increases in soil K^+ and plant K^+ uptake were also demonstrated, which did not occur in the present experiment. It should be noted that

plant biomass analysis was not conducted following the third harvest in 2011, and perhaps the K^+ content of the biochar ash had leached from the char before the first crop of 2012 plants were able to access it.

Increases in P loss through leaching or runoff have been demonstrated in other studies (Lehmann et al., 2003; Schnell et al., 2012), but decreases in P loss have also been demonstrated (Laird et al., 2010; Lehmann, 2007; Novak et al., 2009; Yao et al., 2011). Initial loss of P may have been due to inhibition of P sorption, which has been observed in manure-amended soils and attributed to the production of organic acids during manure decomposition, and the competition between P and organic acids for sorption sites in the soil (Øgaard, 1996). P moves through soil matrix flow rather than macropore flow (Miller et al., 2011), which leads to a slow release from soil over time, thus P leaching from manure peaked with the first rain immediately after application, but continued at lower levels at each subsequent rainfall long after application, as demonstrated in Vadas et al. (2007). Additionally, nutrient release results presented in Chapter 2 indicate that biochar may have a similar release pattern to manure for P. It is also possible that P and K^+ loss were due to top-dressing of manure and biochar and may have been suppressed had the manure and biochar been immediately incorporated thoroughly (Schnell et al., 2012).

These results indicate that biochar incorporated on a large scale may decrease N leaching from agricultural systems (though not likely significantly), and may increase P and K^+ leaching from agricultural systems. Fresh biochar made from wood is not likely to impact greenhouse gas emission or ammonia emission from a high-nutrient input system such as the one described in this experiment.

5.4 Conclusions

Biochar demonstrated the potential to suppress GHG emissions, though treatment differences between the mean values of cumulative N_2O and CH_4 emissions were not significant. pH and total carbon were significantly increased by biochar, indicating some recalcitrance to microbial mineralization of biochar C and a lasting pH effect despite the loss of ash via leaching. NO_3^- intensity was suppressed in HP plots suggesting that nitrification rates were inhibited. Extracts from ion exchange resins indicated that the HB treatment exhibited an increase in cumulative K^+ leaching, as well as a short-term increase in P and NH_4^+ leaching and a short-term decrease in NO_3^- release from manure-amended soils. These data indicate that some types of biochar should be used with caution in systems that include high rates of nutrient application if there is a risk of P loading to surface- or groundwater, unless the ash is removed from the char prior to soil application.

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Chapter 6.
General discussion

6.1 Background

More than one-third of land globally is assigned to grazing and growing of feed for livestock (FAO, 2006). The increasing scale of livestock farming worldwide also produces increasing amounts of manure, which emit GHGs and NH_3 and contribute to nutrient loading to surface- and ground-water via leaching and runoff (FAO, 2006). Biochar is a carbon rich soil amendment (Lehmann and Joseph, 2009) which has demonstrated the potential to suppress GHG emission from soil (Yanai et al., 2007; Spokas et al., 2009; Liu et al., 2011), to sorb NH_3 (Spokas et al., 2011), and to decrease loss of nutrients in leachate (Lehmann et al., 2003; Novak et al., 2009; Knowles et al., 2011). The high environmental impact of livestock production in the very areas that biochar has shown potential may suggest that this would be an ideal system for biochar incorporation. Given that the majority of all agricultural land is dedicated to raising livestock which generate manure, understanding the impact of biochar on manure in storage as well as the impact of biochar following co-application with livestock manure to soil in a high nutrient input system is vital to developing an appropriate deployment plan for biochar on a large-scale basis.

As P and K^+ are limited resources (Askegaard et al., 2004; Cordell et al., 2009) it will become essential to recycle nutrients. An on-farm closed-loop system in which manure and crop residues are used to enrich soils can recycle nutrients to land that grows cattle feed, though some amount of nutrients would ultimately be transferred away from the system as meat or milk. The use of biochar could potentially increase the retention time of nutrients in the soil (through adsorption of nutrients to biochar surfaces) in order to facilitate crop uptake prior to loss via leaching, thereby facilitating the retention of nutrients in the system. In a closed system, biochar made from on-farm wastes such as straw bedding, crop residues, or animal manure would be incorporated into manure and soils, though this was not feasible at the time of these experiments.

This thesis has attempted to assess the retention of dairy manure nutrients following biochar incorporation to soil or manure storages (Chapters 3, 4, and 5), and to evaluate the impact of biochar on GHG and NH_3 emission from dairy manure in storage as well as from soils that have been amended with dairy manure (Chapters 3, 4, and 5). Furthermore, this thesis has assessed the magnitude and dynamics of release of crop nutrients from biochar (Chapter 2) as well from soil that has been amended with biochar (Chapters 3 and 5). Characteristics of the biochar used in this thesis are summarised in Table 1.

Table 1. Characteristics of biochar used in this thesis

Characteristics	Chapters 2 & 3	Chapter 4	Chapter 5	Appendix 1
Feedstock	sycamore	willow chip	waste pine	willow chip
Peak process temperature ^oC	500	450	550	450
Ash %	4.4	--	17	--
pH	9.3	9.3	7.9	8.9
CEC meq/100g	11	22	3.2	--
TOC g kg^{-1}	750	540	710	820
Total N g kg^{-1}	4.7	8.1	9.1	13
PO_4-P mg kg^{-1}	120	--	820	--
K mg kg^{-1}	3300	--	7200	--

-- Not Analyzed

All types of biochar used in this thesis were made from wood-based feedstock to peak temperatures of 450^o to 550^o C, which is a relatively narrow range of peak temperatures compared to other experiments (Spokas and Reicosky, 2009; Yoo and Kang, 2012). Ash content of the sycamore biochar was lower than ash content of the pine biochar, but this fell within the range of other recent field-based experiments (Major et al., 2012; Zhang et al., 2012). The range of TOC, Total N, and other characteristics is also found in other recent field-based experiments (Spokas and Reicosky, 2009; Castaldi et al., 2011; Yoo and Kang, 2012). The effect of biochar source on nutrient cycling in manure-based livestock systems will be evaluated throughout the discussion.

6.2 Nutrient release from biochar

As livestock manure is a source of P and K^+ loss in agricultural systems (Chang et al., 2006; FAO, 2006), it is important to determine whether the addition of biochar can contribute to or suppress loss of these nutrients. The potential patterns of release of P and K^+ over time were illustrated in Chapter 2. Release of P from soil columns amended with fertiliser, manure, or slurry as well as an unamended (soil) control, each with and without biochar, was addressed in Chapter 3, though these results may not accurately reflect P release in the field as plants were not grown in the soil columns. Finally, release of P and K^+ were monitored over 10 months using resin lysimeters in a field experiment described in Chapter 5. Biochar N is not addressed in this section, as biochar feedstock N is volatilised in proportion to C at the time of pyrolysis and the remaining N shares the recalcitrance of the biochar C (Knicker, 2010), whereas P and K^+ are largely conserved, being converted into inorganic forms (thermally mineralised) and retained in biochar in particulate form. Nutrient release findings can be summarised as follows:

- P release from biochar is less in magnitude than K^+ but may potentially be sustained in the field for several seasons.

- K^+ release is higher in magnitude than P but is depleted quickly due to the high solubility of K^+ salts.

- Biochar application temporarily increases PO_4^{3-}-P and K^+ in leachate.

- PO_4^{3-}-P content of biochar that has been applied to soil (particularly when co-applied with manure) is less prone to leaching than manure P.

6.2.1 Phosphorus

Feedstock and biochar analysis in Chapter 2 suggested that P content was retained through pyrolysis, as results reflected the approximate change in mass during pyrolysis. Physical disturbance (crushing) of the biochar contributed to a greater loss of particulate matter over the duration of the experiment as well as a greater release of P. However,

shaking of biochar samples in deionised water did not increase P release over samples that were only soaked in deionised water, which suggests that P was located in particulate form within a different part of the biochar structure than other nutrients (e.g., soluble salts such as K^+). The ratio of P released in the final extraction to the first extraction of the experiment was high, indicating that P release from biochar in a field environment may continue for several seasons.

The concentration of PO_4^{3-}-P in the sycamore biochar that was used in the experiments described in Chapters 2 and 3 was 119 mg kg^{-1}, and was applied in Chapter 3 at a rate of 42 t biochar ha^{-1} or the equivalent of 5.0 kg PO_4^{3-}-P ha^{-1}. Manure in Chapter 3 was applied at 27 t ha^{-1} or 6.4 kg PO_4^{3-}-P ha^{-1} and slurry was applied at 17 t ha^{-1} or 5.1 kg PO_4^{3-}-P ha^{-1}. The release curves of Chapter 2 extrapolated to the biochar application rate utilised in Chapter 3 would have yielded biochar PO_4^{3-}-P release of approx. 1.3-5.5 kg PO_4^{3-}-P ha^{-1}, had leaching of the biochar occurred to the level of completion of release as had occurred over six extractions in Chapter 2. PO_4^{3-}-P release from the biochar-amended treatment which included no other amendments yielded approx. 0.1 kg P ha^{-1} more than the control, as the experiment included only eight 100 mL leaching events for each column of 2 kg soil (dry weight) over 55 days, which was insufficient to replicate the magnitude of release exhibited in Chapter 2. PO_4^{3-}-P release in Chapter 3 was significantly affected by presence of biochar ($p = 0.0325$) and there was also an interactive effect between biochar and nutrient source (fertiliser, slurry, or manure) ($p < 0.0001$). Although the Tukey test results revealed no significant difference between specific treatments, average amounts of PO_4^{3-}-P in leachate from biochar-amended columns for each treatment was greater than that of the (no biochar) counter-parts, with the exception of the manure-amended columns, from which cumulative PO_4^{3-}-P in leachate was higher than the treatment that included both manure and biochar. Interestingly, soil extraction results indicated that columns amended with both manure and biochar demonstrated the greatest decrease in soil PO_4^{3-}-P over the duration of the experiment, indicating either that biochar had facilitated the transformation of manure PO_4^{3-}-P to organic P, or that manure organophosphates adsorbed to biochar surfaces were no longer extractable. Furthermore,

in Chapter 3, manure-amended columns, manure and biochar-amended columns, slurry amended columns, and slurry and biochar amended columns leached approx. 13.5%, 4.7%, 6.2%, and 4.5% of applied PO_4^{3-}-P, respectively, indicating that either biochar PO_4^{3-}-P is protected from leaching from within the biochar structure, or that the presence of biochar has suppressed the release of manure P. The inclusion of plants in this study may have impacted P results due to plant uptake, and any effect of biochar on plant growth or root structure may have further affected results.

The concentration of PO_4^{3-}-P in the biochar that was described in Chapter 5 was 820 mg kg^{-1}, or 6.8 times the PO_4^{3-}-P content of the sycamore biochar used in Chapters 2 and 3, which was applied at a rate of 18.8 t biochar ha^{-1} in the high-application treatment. This was the equivalent of 15.4 kg PO_4^{3-}-P ha^{-1}, or 3.1 times the rate of biochar PO_4^{3-}-P application in Chapter 3. Manure application rates in this experiment were 151.4 m^3 ha^{-1}, or 5.1 kg PO_4^{3-}-P ha^{-1}. These manure application rates were much higher than manure application rates in Chapter 3, though manure PO_4^{3-}-P application rates were similar between these two experiments due to the low PO_4^{3-}-P concentration of the manure used in Chapter 5. The average crop uptake of total P from one harvest in Chapter 5 was approx. 31 kg ha^{-1}. Although there was no statistically significant difference between mean values of cumulative P in leachate in Chapter 5, approx. 1.8 kg PO_4^{3-}-P ha^{-1} more was leached rom the high application biochar plots than the control over the course of 10 months. This occurred primarily in the month of the first rainfall following manure and biochar application, at which time there was a significant difference between treatments, and the high biochar application plots leached approx. 1.7 kg PO_4^{3-}-P ha^{-1} more than the control. There was also a significant difference between high application plots and the control during a heavy rainfall event in late January, which was the highest daily rainfall of the year and followed an extended (> 30 days) drought period. Approx. 72% and 27% of applied (manure and biochar) PO_4^{3-}P was leached from the control and high application biochar plots, indicating (as in Chapter 3) that biochar PO_4^{3-}-P is not as accessible to soil solution, that the presence of biochar stimulates the transformation of mineral P to organic P, and/or that biochar adsorbs manure P. Although 30-103% of the

total biochar P was removed during six deionised water extractions in Chapter 2, the soil column and field-based results suggest that the PO_4^{3-}-P content of biochar that has been applied to soil, particularly when co-applied with manure, is less prone to leaching than manure P. Initial loss of P following land application may be due to inhibition of P adsorption, which has been observed in manure-amended soil and attributed to the production of organic acids during manure decomposition, and the competition between P and organic acids for sorption sites in the soil (Øgaard, 1996). However, over time it is possible that a greater amount of manure P was transformed through microbial synthesis to organic P in biochar amended soil, as organic amendments are favoured in the microbial synthesis of organic P (Malik et al., 2012). Additionally, orthophosphate and organic P compounds may sorb to biochar surfaces, as suggested Laird et al. (2010) and Novak et al. (2009).

Overall, any notable increase in PO_4^{3-}-P leaching from wood-derived (low P) biochar (such as the biochar used in each experiment in this thesis) applied to grassland soil that receive high manure inputs is likely to be short-term and effects on cumulative annual leaching are not likely to be significant. Furthermore, the use of a similar biochar application rate in a field environment in the UK may have yielded different results as manure application rates and timing are restricted by regulations such as the European Union Nitrates Directive (European Commission, 1991). Biochar containing higher amounts of P may have induced significant long-term leaching of P from the study site. Further research is required assessing biochar that contains a high amount of P, such as biochar derived from poultry manure (Hass et al., 2012).

Based on the P results presented in this thesis, the hypothesis that biochar incorporated into sandy soils used to grow ryegrasses for silage will decrease nutrient leaching (with respect to P leaching) following manure application is rejected.

6.2.2 Potassium

Feedstock and biochar analysis in Chapter 2 indicated that approx. half of the K^+ content of the feedstock was lost or not easily extractable following pyrolysis. Although K^+ concentrations in this type of biochar were 3.3 g K^+ kg^{-1} biochar, six extractions yielded cumulative values of approx. 2.4-4.5 g K^+ kg^{-1} biochar, indicating that a higher proportion of the feedstock K^+ may still be present. A different method of analysis such as ashing and borate fusion (Wu et al. 2011) or modified dry ashing (Enders and Lehmann, 2012) may have yielded results reflecting a higher K^+ concentration in the biochar.

Physical disturbance (crushing) as well as shaking (versus soaking) led to greater loss of K^+, most likely due to greater movement of water into smaller pore spaces. The high release rate constant of K^+ and the relatively low ratio of release of the sixth extraction to that of the first extraction indicated that the water-available portion of K was released very quickly due to the high solubility of K^+-containing salts.

In Chapter 5, manure was applied at a rate of 389 kg K^+ ha^{-1} and biochar was applied to the high application plots at a rate equivalent to 135 kg K^+ ha^{-1}. Although K^+ leaching from UK agriculture has been documented at rates of 20-50 kg ha^{-1} y^{-1} (Askegaard et al., 2004), the amount of K^+ leaching that was documented in Chapter 5 was approx. 169-257 kg ha^{-1} over 10 months. Given the high manure application rate used in this California field site compared to rates that are typical in the UK, and given the high solubility of K^+, this is not surprising. Also the high ash content of biochar used in Chapter 5 (17%) was likely to have contained high concentrations of multivalent cations such as calcium (Ca^{2+}), which would have preferentially occupied soil and biochar exchange sites and induced an increase in K^+ leaching. A mass balance analysis conducted by Laird et al. (2010) indicated that the presence of biochar decreased the leaching of manure K^+ due to the influence of combined effects of multiple K^+ sources as well as an influence of biochar on the mobility of K^+. This may have been reflected in Chapter 5 following the highest daily rainfall event of the year which occurred in late January after a month-long drought, at which point significantly less K^+ was leached from

the low application biochar plots than the control. However, biochar is unlikely to have a long-term effect on manure K^+ as there was no significant difference between treatments when the amount of leached K^+ was expressed as a proportion of applied K^+.

As nearly half of the K^+ applied as manure and biochar to the high application biochar plots was leached over 10 months, it is unlikely that a significant amount of K^+ from biochar application to soil would be available beyond one to two growing seasons following application. However, should any concern arise about increased leaching of K^+ from biochar-amended sites, this also indicates that the increase (assuming a one-time application) would be short term. Furthermore, selection of biochar with a low K^+ content such as biochar made from greenwaste (Cox et al., 2012) may decrease the risk of elevated K^+ loss in leachate following biochar application. Additionally, the use of a similar biochar application rate in a field environment in the UK may have yielded different results as manure application rates and timing are restricted by regulations such as the European Union Nitrates Directive (European Commission, 1991).

Although K^+ is not considered to be a pollutant, it has been documented that crop uptake of K^+ can exceed crop requirements, and that high concentrations of K^+ in forages can lead to depressed dietary magnesium (Mg^{2+}) and Ca^{2+} levels in cattle, thereby affecting animal health and potentially causing death of cattle (Chang et al., 2006). A K/(Ca+Mg) charge ratio exceeding 2.2 in forages is commonly considered a risk factor for grass tetany (Rendig and Grunes, 1979). Although Ca^{2+} and Mg^{2+} concentrations in grasses were not analysed in Chapter 5, biochar application to soils did not increase K^+ concentration in grass tissues. However, K^+ uptake in grassland systems is important and should continue to be monitored in future biochar studies.

Based on the K^+ results presented in this thesis, the hypothesis that biochar incorporated into sandy soils used to grow ryegrasses for silage will decrease nutrient leaching (with respect to K^+ leaching) following manure application is rejected.

6.3 Nitrogen dynamics in manure and soils

Dairy manure is an important source of N to soil, but can contribute to NO_3^- leaching to ground- and surface-water (FAO, 2006; Moore et al., 2011). It has been well documented that biochar affects nitrogen dynamics within soils (Clough and Condron, 2010) through direct adsorption of mineral N (Dempster et al., 2012) as well as through microbial immobilisation (Nelissen et al., 2012) and other influences on soil microbial communities (Dempster et al., 2012), possibly due to the evolution of ethylene (Spokas, 2010) or the potentially toxic volatile organic compounds present on the biochar (Deenik et al., 2010). The effect of biochar on N dynamics of manure in storage had not yet been explored. Soil nitrogen was monitored in two incubation experiments. The first addressed the impact of biochar on nutrient retention in soils amended with diverse nutrient sources (Chapter 3) and the second addressed the impact of biochar on soil N under fluctuating moisture regimes (Appendix 1), though neither of these experiments utilised plants which would have impacted the results. Furthermore, soil N was monitored for one year in a field-based experiment which addressed the impact of biochar on nutrient retention following manure addition to a field used to grow ryegrasses for silage (Chapter 5). The effect of biochar on manure N in storage was discussed in Chapter 4. Additionally, Chapter 3 and Chapter 5 report monitoring of the release of NO_3^- and NH_4^+ in leachate. Findings related to the impact of biochar on N dynamics can be summarised as follows:

- Biochar applied as a cap to cattle slurry in storage decreased depletion of the NH_4^+ pool.

- Biochar significantly decreased mineral N in leachate from the nutrient-poor sandy soil used in the incubation study and temporarily decreased mineral N in leachate from the nutrient-rich sandy soil in the field experiment.

- Results from all soil incubation studies and the field study suggest that biochar may suppress the nitrification process in soils.

Biochar applied as a cap to cattle slurry in storage (Chapter 4) decreased depletion of the NH_4^+ pool over the course of the experiment. This was reflected both in slurry NH_4^+

concentration for this treatment, which were higher than all other treatments, and slurry NO_3^-, which was below the detection limit. This indicates treatment effects on nitrification, immobilisation, and/or ammonia volatilisation. However this may also have been partly due to the impact of high emission of NH_3 on nitrite oxidisers (Villaverde et al., 1997; Clough et al., 2010). Conversely, biochar that had been thoroughly mixed into cattle slurry in storage demonstrated the lowest average NH_4^+ concentrations of all treatments as well as the highest NO_3^-. This indicates either an enhancement of nitrification or a suppression of denitrification, possibly through adsorption of NO_3^- to biochar.

The results of Chapter 3 indicated that change in soil mineral N was significantly affected by N source ($p < 0.0001$) and that there was an interaction between N source and biochar ($p = 0.0150$). The presence of biochar may have had a weak effect ($p = 0.0593$). At the same time, biochar significantly decreased ($p < 0.0001$) cumulative mineral N in leachate and cumulative results were lower for both NO_3^- and NH_4^+ concentrations in leachate from biochar amended soil. This suggests that biochar either promoted biotic immobilisation, or facilitated the accumulation of NH_4^+ by chemical adsorption or the suppression of nitrification. It has been demonstrated that biochar can impact the nitrification process (Clough et al., 2010), possibly due to hydrocarbons sorbed to biochar having an initial effect of suppression of soil microbial communities (Spokas et al., 2009). An impact on nitrite oxidisers due to NH_3 volatisation was not likely, as N sources that are injected or incorporated into soil emit little to no NH_3 (Rodhe et al., 2006). In Chapter 3, an impact on the nitrification process is reflected in greater NH_4^+ retention in biochar amended soils (reflected in the soil change ratios) and the higher NO_3^- concentrations in leachates from soil that had not been amended with biochar, though other studies have reported no inhibition of nitrification rates (Singh et al., 2010; Bruun et al., 2011). The inclusion of plants in this study may have impacted N results due to plant uptake, and any effect of biochar on plant growth or root structure may have further affected results.

An impact of biochar on the nitrification process was also reflected in the low soil NO_3^- intensity values of high-application biochar amended plots in Chapter 5, as well as the temporary increase of leachate NH_4^+ from high application plots above that of the control following the first rain event, and the temporary decrease of leachate NO_3^- from high application plots below that of the control following the highest daily rainfall event in late January. Likewise, biochar significantly decreased soil NO_3^- in Appendix 1 ($p < 0.0001$), indicating the suppression of the nitrification process. Decreased soil NO_3^- has been reported by others (Nelson et al., 2011; Case et al., 2012; Sarkhot et al., 2012; Taghizadeh-Toosi et al., 2012), as has a decrease in nitrification rates (Dempster et al., 2012; Sarkhot et al., 2012), which is considered to be a result of the increased C to N ratio (Burgos et al., 2006; Andersen and Petersen, 2009) or due to adsorption of NO_3^- to biochar surfaces (Joseph et al., 2010). However, in Appendix 1, NH_4^+ was also significantly decreased by the presence of biochar ($p = 0.0014$) suggesting biotic immobilisation or that N mineralisation may have been suppressed. A simultaneous impact of biochar on the mineralisation and nitrification rates was reported by Dempster et al. (2012), and lower NH_4^+ concentrations in biochar amended soils have been reported by others (Castaldi et al., 2011; Sarkhot et al., 2012; Taghizadeh-Toosi et al., 2012). However, it should be noted that the presence of biochar can affect the NH_4^+ extraction process, as was reflected in the day 0 sample results of Chapter 3, and low NH_4^+ results in biochar amended soils may be an artefact of this effect.

Although Chapter 3 indicates that biochar has a high potential to increase nitrogen retention in poor soil, the results of Chapter 5 suggest that effects of pine chip biochar on N retention in a grassland soil with a high annual manure application rate are likely to be short term and not significant in terms of cumulative annual N loss. However, application of pine chip biochar to a soil that was more similar to that used in the incubation studies (or to a grassland system in the UK with lower annual manure application rates) may have yielded more favourable results. It is also possible that application of a sycamore biochar (similar in pH, CEC, etc. to that used in chapters 2 and 3) to the California field system may have yielded more favourable results than those following application of the pine

chip biochar. Chapter 5 results were consistent with Schulz and Glaser (2012), who reported that biochar addition changed N dynamics in soil whilst total N retention was not influenced. Kameyama et al. (2012) reported that long term NO_3^- retention is unlikely as it is weakly adsorbed by biochar and then desorbed following water infiltration, however the presence of biochar could increase the residence time of NO_3^- in the root zone.

Based on the N results presented in this thesis, the hypothesis that biochar incorporated into sandy soils used to grow ryegrasses for silage will decrease nutrient leaching (with respect to N leaching) following manure application is neither accepted nor rejected as the impact of biochar on N leaching is dependent on soil type, biochar type, and N application rates, among other factors.

6.4 Greenhouse gas and ammonia emission

Dairy systems are an important source of NH_3 and GHGs (FAO, 2006), both from manure storage as well as soils after manure and fertiliser application. Biochar has demonstrated the potential to suppress NH_3 emission (Taghizadeh-Toosi et al., 2012) as well as greenhouse gas emission (Liu et al., 2011; Sarkhot et al., 2012) from soil. The impact of biochar on NH_3 and GHG (CO_2, CH_4, and N_2O) emission from manure in storage was addressed in Chapter 4, whilst the impact of biochar on NH_3, N_2O, and CH_4 emission from manure applied to a field used to grow ryegrasses for silage was addressed in Chapter 5. Additionally, soil column experiments explored the effect of biochar on N_2O emission from soils amended with diverse nutrient sources including manure and slurry (Chapter 3) as well as soils under a fluctuating moisture regime (Appendix 1). Findings related to the impact of biochar on NH_3 and GHG emission can be summarised as follows:

- There was no statistically significant impact of biochar on NH_3 emission from soil amended with manure.

- There was no statistically significant impact of biochar incorporated into slurry in storage on NH_3 emission; biochar applied as a cap to slurry in storage significantly increased NH_3 emission.

- The effect of biochar on N_2O emission from soil varied between experiments.

- Biochar incorporated into slurry in storage or applied as a cap significantly decreases N_2O emission from slurry.

- There was no statistically significant impact of biochar on CH_4 emission from soil amended with manure.

- Biochar incorporated into slurry in storage significantly increases CH_4 emission from slurry; biochar applied as a cap to slurry in storage has no significant impact on CH_4 emission from slurry.

6.4.1 Ammonia

In Chapter 5, biochar-amended plots emitted less NH_3 than the control, however the treatment effect was not statistically significant. It is possible that a higher biochar application rate would have been more effective. For example, in Taghizadeh-Toosi et al., (2012), pine based biochar was applied at a rate of 30 t ha^{-1}, and NH_3 emission was significantly suppressed in relation to the control. As the biochar in Chapter 3 increased NH_3 emission, biochar with a higher pH than the biochar used in the field experiment (such as those used in the incubation experiments) may not be effective. Acidified biochar may have been more efficient at suppressing NH_3 emission (Doydora et al., 2011) and short-term acidification of soil due to amendment addition has demonstrated potential to suppress N_2O (Baggs et al., 2010; Fangueiro et al., 2010). However, this could potentially have adverse effects such as increased P leaching or impacts on plant health (Doydora et al., 2011).

In Chapter 4 a high rate of biochar (2:1 slurry to biochar, based on dry weight) mixed into slurry that was in storage emitted less NH_3 than all other treatments, but (as in Chapter 5) this rate of emission was not significantly less than the control. Furthermore, biochar applied as a cap to the surface of the slurry significantly increased NH_3 emission above that of all other treatments, indicating that the liming effect of biochar (Chan et al., 2007) could enhance NH_3 emission from biochar amended manure. However, overall emission factors when expressed as a percentage of slurry N were less than 0.01% for all treatments, though this may have been an artefact due to the use of a closed system rather than a through flow system (Sherlock et al., 2002). Here again, the use of acidified biochar may have had a significant impact on NH_3 emission.

Based on the NH_3 results presented in this thesis, the hypothesis that biochar incorporated into sandy soils used to grow ryegrasses for silage will decrease NH_3 emission following manure application is rejected. Furthermore, the hypotheses that biochar incorporated into slurry that is in storage or applied as a cap to slurry that is in storage will decrease slurry NH_3 emission are also rejected.

6.4.2 Nitrous oxide

The effect of biochar on N_2O emissions varied between experiments. Wood-derived biochar heated to peak temperatures of 450-550ºC was used in all experiments. Sycamore biochar and willow biochar decreased N_2O emissions from soil in Chapter 3 and from slurry in Chapter 4 (respectively), but pine biochar and willow biochar did not significantly impact N_2O emission from soil in Chapter 5 or Appendix 1 (respectively). As the soil that was used in Appendix 1 was from the same site described in Chapter 3 with a biochar application rate identical to that described in Chapter 3, and as the biochar that did not suppress emission in Appendix 1 was made from the same feedstock, within the same pyrolysis oven, and to the same peak process temperature as the biochar that suppressed N_2O emission from slurry in Chapter 4, the effectiveness of willow chip biochar in Chapter 4 was likely to have been at least partly attributable to its effect on slurry pH. Prior to the start of the experiment described in Appendix 1 the biochar was leached six times (similar to the biochar samples used in the experiment in Chapter 2), thereby removing ash content and limiting the liming effect of the biochar. Although the pH values of biochar amended columns in Appendix 1 were significantly higher than those that were not amended with biochar ($p < 0.0001$), it is likely that the magnitude of this effect was mitigated by the pretreatment of biochar. Had the experiment described in Appendix 1 included treatments that incorporated unleached biochar, the effect of biochar ash content on N_2O emission in this study may have been conclusive. Additionally, suppression of N_2O emission from the high application biochar treatment (2:1 slurry to biochar based on dry weight) in Chapter 4 was partially attributed to NO_3^- adsorption as fresh biochar can have a positive charge (Cheng et al., 2008b). However, unlike the soil columns in Chapter 3 in which soil and all amendments were thoroughly mixed, biochar in Appendix 1 was thoroughly incorporated into the soil whilst fertiliser was applied to the soil surface, thereby potentially limiting the physical contact and proximity between biochar surfaces and the NO_3^- and NH_4^+ ions. Furthermore, the inclusion of plants in either incubation study may have impacted N_2O results due to plant

uptake of N, and any effect of biochar on plant growth or root structure may have further affected results.

Although the field experiment described in Chapter 5 demonstrated the potential for N_2O suppression, cumulative N_2O emissions from biochar amended plots were not significantly different from the control. The lack of a significant treatment effect may have been due to spatial variability, as has been reported in other field-based studies (Chadwick et al., 2000; Wulf et al., 2002; Fangueiro et al., 2008), though it is possible that the use of the pine chip biochar on a soil that was more similar to those used in the incubation experiments may have had a significant impact on N_2O emission. It is also possible that application of a sycamore biochar (similar in pH, CEC, etc. to that used in chapters 2 and 3) to the California field system may have yielded more favourable results than those following application of the pine chip biochar. If continued over several seasons, pine chip biochar in the California field system may have demonstrated a significant effect on cumulative emissions over time, possibly due to nitrification inhibition as evidenced by the treatment effect on soil NO_3^-. Though pine based biochar has suppressed N_2O emission in a ryegrass field setting, this occurred with higher biochar application rates of 30 t ha^{-1} (Taghizadeh-Toosi et al., 2011), versus the application rate of 18.8 t ha^{-1} that was implemented in Chapter 5. Urea was applied at a much higher rate in Taghizadeh-Toosi et al. (2011), though only within the gas sampling chambers, at 930 kg N ha^{-1}, which was higher than the application rate of 409 kg N ha^{-1} in Chapter 5. Fertiliser application to the rest of the field was a modest 133 kg ha^{-1} divided into two application events, suggesting that annual application rates of nutrients may have been much lower than in Chapter 5. Furthermore, the soil in Taghizadeh-Toosi et al. (2011) was a finer texture with a lower pH (5.5) than the soil in Chapter 5 (7.3), indicating that there was a higher potential for the pine biochar in Taghizadeh-Toosi et al. (2011) to impact soil pH and aeration than the conditions present in Chapter 5.

Although biochar has demonstrated the potential to suppress N_2O emission from manure- and slurry-amended soils, a stronger potential has been exhibited in nutrient poor soils

with low pH values, such as the soil utilised in Chapter 3. Biochar application to field soils with the intention of N_2O suppression would most likely require high biochar application rates (equal to or greater than 30 t ha^{-1}) and co-incorporation of manure and amendments into the soil profile may aid in adsorption of N to biochar surfaces. Additionally, if the liming effect of biochar ash content is a key factor in the suppression of N_2O emission from a specific soil type, the biochar may not be effective in N_2O suppression beyond one or two seasons.

Biochar application to manure prior to land application has demonstrated a strong potential for suppression of N_2O emission, though this is only required in cases of long-term storage (> 30 days) in which there is minimal disturbance and sufficient time for a crust to form on the surface of the slurry.

Based on the N_2O results presented in this thesis, the hypothesis that biochar incorporated into sandy soils used to grow ryegrasses for silage will decrease GHG emission (with respect to N_2O emission) following manure application is neither accepted nor rejected, as impact on N_2O emission is still not well understood and depends largely on soil type, biochar type, and N application rate, among other factors. However, the hypotheses that biochar incorporated into slurry that is in storage (given a high biochar application rate) or applied as a cap to slurry that is in storage will decrease slurry GHG emission (with respect to N_2O emission) are accepted.

6.4.3 Methane

Although biochar has demonstrated the potential to suppress CH_4 emission from soils (Yoo and Kang, 2012), CH_4 suppression was not demonstrated in experimental results contained in Chapters 4 and 5. Although average cumulative CH_4 emissions from high application biochar amended plots in Chapter 5 were 61% of those of the control, these differences were not significant, thereby highlighting the challenge of high spatial variability in field-based experiments. Additionally, as potential treatment effects did not

begin to become apparent until the biochar had been present in the soil for over 9 months, a longer period of study may have yielded results with significant treatment effects.

CH_4 emission from biochar amended slurry (2:1 ratio slurry to biochar, based on dry weight) was significantly higher than that of the control. Given that suppression of CO_2 and N_2O emission was observed in the same treatment, an impact on the slurry microbial communities including the methanotroph population can be inferred. Given the high global warming potential of CH_4, biochar should not be used when applied to slurry in short-term storage (< 30 days) or slurry that is continuously agitated thereby hindering the formation of a crust.

Based on the CH_4 results presented in this thesis, the hypothesis that biochar incorporated into sandy soils used to grow ryegrasses for silage will decrease GHG emission (with respect to CH_4 emission) following manure application is rejected. Furthermore, the hypotheses that biochar incorporated into slurry that is in storage or applied as a cap to slurry that is in storage will decrease slurry GHG emission (with respect to CH_4 emission) are also rejected. However, given the high global warming potential of N_2O and the impact of biochar on N_2O emission from slurry described in the previous section, biochar incorporated into slurry that is in storage or applied as a cap to slurry that is in storage may potentially decrease overall slurry GHG emission (with respect to CO_2-equivalent emission).

6.5 Future challenges and conclusions

The work in this thesis has underlined some of the most important questions regarding the incorporation of biochar into grassland and dairy systems, however it has also highlighted some of the shortcomings of biochar research to date.

Overall, these results combined with the results of others indicate that whilst biochar is highly stable as a carbon sequestration technology and may be useful in the future development of carbon credits, it may be problematic to include suppression of trace gases in the carbon accounting of a biochar-amended system as the impact of biochar on GHG emissions varies widely depending on type of biochar, soil type, and other factors. Furthermore, choice of biochar is important in high nutrient input systems as biochar can add to nutrient release to ground- and surface-water, though the differences in nutrient release patterns between different biochar types is not well understood. The use of biochar with a lower ash content than those used in this thesis may reduce risk of increased leaching of nutrients from soils following biochar addition. Use of biochar in systems with high nutrient input such as California dairy systems should not be adopted until these uncertainties are addressed, though use of biochar in poorer soils such as those used in the incubation experiments may yield more favourable results. Furthermore, barring the development of a carbon credit, farmers are unlikely to use biochar in a system such as this unless a consistent suppression of GHG and NH_3 emission or nutrient loss via leaching is demonstrated. Additionally, if the benefits of a mixture of manure and biochar as a soil amendment are demonstrated on soils with a lower annual rate of nutrient input than those typical at a dairy farm with a high stocking density, dairy farmers may choose to create a product that is moved off-farm, thereby opening the system to surrounding areas.

These results also indicate that adoption of biochar co-incorporation with manure in systems with nutrient-poor soil such as those used in the incubation experiments would be likely to contribute to greater benefits in terms of plant growth, GHG suppression, and

nutrient retention than systems such as the one highlighted in Chapter 5. Further field-based research should be conducted in areas of the world in which nutrient-poor soils are commonplace.

The effect of biochar on GHG emission and nutrient leaching from soil has been the subject of more than 100 published studies, however there has been no conclusive evidence of established mechanisms for GHG suppression and nutrient retention and there has also been a lack of predictability and consistency of results. Furthermore, it is likely that several mechanisms contribute to suppression of GHG emission from biochar amended soils, though the dominant mechanism(s) may differ between experiments due to differences in pH, moisture regime, temperature, and soil and biochar types. Due to a lack of readily available biochar as well as constraints on time and other resources in an academic environment, most experiments have focused on one biochar type and one soil type over a short timeframe (usually one year or less). If indeed there is interest in the use of biochar within a high nutrient input system such as the dairy systems of California, incubation studies with a soil type and rate of nutrient addition that reflect this environment should be conducted with several types of biochar that include a range of ash contents, perhaps with ash removed prior to use. Using biochar with a defined range of physical characteristics, possibly with the inclusion of an acidified biochar to assess the effects on NH_3 and N_2O emissions, as well as the effects on nutrient leaching could be important. Furthermore, long term field-based biochar studies would help assess the impact of biochar on nutrient retention once a large proportion of the nutrient content of the biochar has been leached away and the CEC of the biochar increased due to biotic and abiotic oxidation (Cheng et al., 2006; Liang et al., 2006; Cheng et al., 2008a).

There have been a number of studies regarding the effect of biochar on N_2O emission from soil specifically. As biochar has shown a general impact on soil microbial communities, future studies could assess the effects on their diversity and structure, as well as size before and after biochar addition. These studies could utilise fumigation extraction, DNA extraction, and functional gene analysis. Similar to Ollivier et al. (2010)

which explored the effect of manure from antibiotic-treated animals on soil microorganisms, a biochar study could include *nifH*, *amoA* (to assess ammonia-oxidising bacteria and archaea), *nirK*, *nirS*, and *nosZ* (to assess denitrification). Nitric acid reductases (*qnorB*) could also be assessed (Pastorelli et al., 2011). Furthermore, these studies could be conducted using several types of biochar with a range of labile carbon contents, in order to assess the impact of biochar on microbial immobilisation, and volatile organic carbon contents, in order to assess the impact of biochar on microbial activity due to presence of toxins, perhaps focusing on specific toxins, such as naphthalene and fluorine which are commonly found in biochar (Kloss et al., 2012). Future incubation and field studies could incorporate ^{15}N-labelled manure in order to differentiate between soil N and manure N, as well as potentially determine if N_2O suppression from biochar is due to enhanced denitrification directly to N_2 rather than due to nutrient retention within the soil. Similarly, ^{32}P could be utilised in order to differentiate between biochar P and fertiliser or manure P that is lost in leachate or taken up by biomass, as demonstrated in ^{32}P studies conducted by Xie et al. (2004) and Shrivastava et al. (2011). Studies such as these could clarify the release pattern of biochar P as well as the effect of biochar on the retention or release of manure P.

Further research could also be conducted to address the impact of biochar that has been ingested by cattle during grazing, particularly with regard to the polyaromanic hydrocarbon content of the biochar.

Additionally, a field study could be conducted to address the impact of the transportation of a manure-biochar mixture from the high input system of a grassland dairy farm to a lower input system such as a neighbouring vineyard or arable farm, particularly if the soils are highly weathered or nutrient poor. The use of a site such as this, particularly in California where stocking densities on dairy farms are high, could allow manure application to grasslands to decrease to levels such as those recommended in the UK (DEFRA 2010), as well as potentially increase the impact of biochar on a system that is more likely to benefit from addition of manure and biochar. Such a system could utilise

biochar made from crop wastes (for example, vineyard clippings), providing a waste management solution for multiple farmers as well as exploring a potentially more suitable environment for these amendments.

This thesis has highlighted many important aspects of biochar and its potential effect in a grassland or dairy farm. Although the time constraints of this programme have prevented measurements over a period of time greater than one year, there were still significant findings that have highlighted the areas in which further study could be beneficial, and have perhaps paved the way for more specified experiment design in the area of grassland systems. This thesis has indicated that there exists potential for biochar to decrease N_2O emission and increase nutrient retention, particularly from manures that are in storage for more than 30 days, as well as soils with low nutrient and carbon contents. This thesis has also highlighted areas in which careful selection of biochar would be important, for instance application of biochar to manure in storage for less than 30 days, or application to areas to which there is a high K^+ input and potential for enhanced K^+ uptake from forages which could be dangerous to cattle, as well as areas from which there is a risk of P loading to surface and ground-water.

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Appendix 1.

‘Establishing the impact of biochar on soil N_2O emissions under a fluctuating soil moisture regime’

Introduction

This material is supplementary and is not considered to be part of the main body of this thesis. This is a partial manuscript which includes materials and methods as well as results to be referred to in Chapter 6. This manuscript will be completed at a later date.

Materials and methods

The present study was conducted using soil columns in a controlled temperature room at the UK Biochar Research Centre at University of Edinburgh, Scotland, UK. The room was maintained at 30ºC. Sandy loam soil was obtained in July 2010 from the Rothamsted Research Woburn Experimental Station, Woburn, Bedfordshire, UK. This soil has not been cultivated for approximately 60 years resulting in low total organic carbon (TOC) (4.0 g kg^{-1}) and total N (247 mg kg^{-1}) with a pH of 6.03 (in deionised water). After collection, the soil was passed through a 6 mm mesh sieve.

The biochar was made from willow chips (*Salix* spp.) heated from ambient temperature (8°C) to 450ºC over the course of 60 minutes and maintained at 450ºC for 29 minutes using a batch process slow pyrolysis unit with a water-cooled condenser, an internal auger for feedstock agitation during the reaction process, and an internal thermocouple which measures the temperature in the core of the combustion chamber and controls the process. The biochar contained 822 g kg^{-1} C and 13.2 g kg^{-1} N with a pH of 8.87. The biochar was manually ground and sieved to a 1.18-4.00 mm particle size range, subjected to six serial four-hour soaking events in deionised water (20:1 water to biochar ratio by mass) in order to reduce the ash content of the biochar, and dried for 24 h in an oven at 105ºC.

There were four treatments with four replicates, randomized spatially (no blocking) within the controlled temperature room. Treatment codes were assigned as follows: control (soil only) that was kept saturated (CW), soil plus biochar that was kept saturated (BW), control (soil only) that was subject to wetting and drying cycles (CWD), and soil plus biochar that was subject to wetting and drying cycles (BWD). At the start of the experiment, 16 (each 900 g dry weight) soil samples were prepared. Soil for each replicate column was prepared separately, being weighed out and then mixed with biochar where applicable, using a trowel. Biochar that had been dried for 24 hours at 105ºC was mixed into the soil at a rate of 2% based on dry weight. Deionised water was added on a 1:1 ratio (dry mass basis) to the biochar prior to addition to the soil in order to avoid the

biochar drawing water from the bulk soil after addition. This was in recognition of the fact that only part of the capacity of biochar to hold water outside the soil is available after addition.

The soil for each sample was transferred to 35 cm long columns made from 11 cm diameter polyvinyl chloride piping. The columns were packed evenly by hand to a depth of approx. 7 cm (no biochar amendment) or 7.5 cm (for columns that contained biochar), based on the bulk density of soil in Chapter 3. The base of each column was lined with two layers of nylon mesh and one layer of cotton muslin, clamped to the column by a pipe connector that also served to elevate the soil and cloth liner from the table surface approx. 5 cm.

Approximately 0.06 g NH_4NO_3 at an equivalent to the area-based rate of 22 kg N ha^{-1} (74 N kg ha^{-1}, assuming a 23 cm plough depth) was added slowly to each column in a dilute solution with deionised water in order to bring the soil to 100% gravimetric water holding capacity (WHC). WHC was calculated using the data generated in Chapter 3. Samples were then placed randomly throughout the controlled temperature room at 30ºC for 45 days to facilitate drying of the soil. One CW column was disturbed on day 7 and omitted from statistical analyses.

All columns were weighed daily. The CW and BW columns were adjusted to 100% WHC daily using deionised water based on weight. The CWD and BWD columns were adjusted to 100% WHC on days 9, 18, 27, and 36, prior to which the moisture content had decreased to approx. 21% WHC.

On each re-wetting occasion (days 9, 18, 27, and 36), an additional 0.06 g NH_4NO_3 was added to each of the 16 columns, in a dilute solution of deionised water equal to the amount required to bring each column to 100% WHC. Total NH_4NO_3 addition throughout the course of the experiment was approx. 0.30 g to each column, equivalent to the area-based rate of 111 kg N ha^{-1} (366 kg ha^{-1}, assuming a 23 cm plough depth).

Headspace gas samples were taken daily with the exception of day 44. At the beginning of each gas sampling event, 50 mL of water were added to the leachate collection dish to create an air-tight seal below the column. Headspace chambers comprising 25 cm sections of additional piping with airtight lid, sampling port and 3-way stopcock were connected to the top of each column with a rubber ring and the time was recorded. After 30 min closure a headspace gas sample was taken for each column using a vacuum hand pump and a 20-22 mL glass vial sealed with a crimp cap and chloro butyl rubber septum (Chromacol, Welwyn Garden City, UK). The vials were filled from the headspace and evacuated to 70 kPa twice prior to keeping the third sample for analysis. Two ambient air samples were taken within the incubation room at each sampling event. Duplicate samples were taken from two columns during each event and an analysis of linearity of N_2O emissions was conducted weekly. Gas samples were analysed for N_2O using an Agilent 6890 Series Gas Chromatograph (Santa Clara, CA, USA). Linearity analysis was conducted by sampling from one column once every 5 min for the period of 1 h. The results were plotted and relationships analysed by linear regression.

Soil samples were taken from each column after dismantling on day 45. The soil was thoroughly mixed before the sample was taken. pH of soil samples was determined by adding deionised water in a 2:1 ratio, shaking the sample for 30 min, and using a Thermo Russell RL150 pH Meter (ThermoFisher Scientific, Weltham, Massachusetts, USA). NH_4^+ and NO_3^- concentrations were quantified through 1 N KCl extraction and determined colorimetrically using an autoanalyser (Bran and Luebbe AA3, Seal Analytical, Norderstedt, Germany). Additionally, soil samples were milled using a Retsch ball mill and total C and N concentrations were determined using a Carbo Erba 2500 Elemental Analyser (Rodano, Italy).

Calculation of cumulative results and statistical analysis

Cumulative N_2O emissions were estimated using the linear trapezoidal method. Average pH results for leachate and soil samples were calculated by converting pH results to $[H^+]$,

calculating the arithmetic mean, and converting the mean back to pH. Variables were summarized using arithmetic means and standard errors (SE).

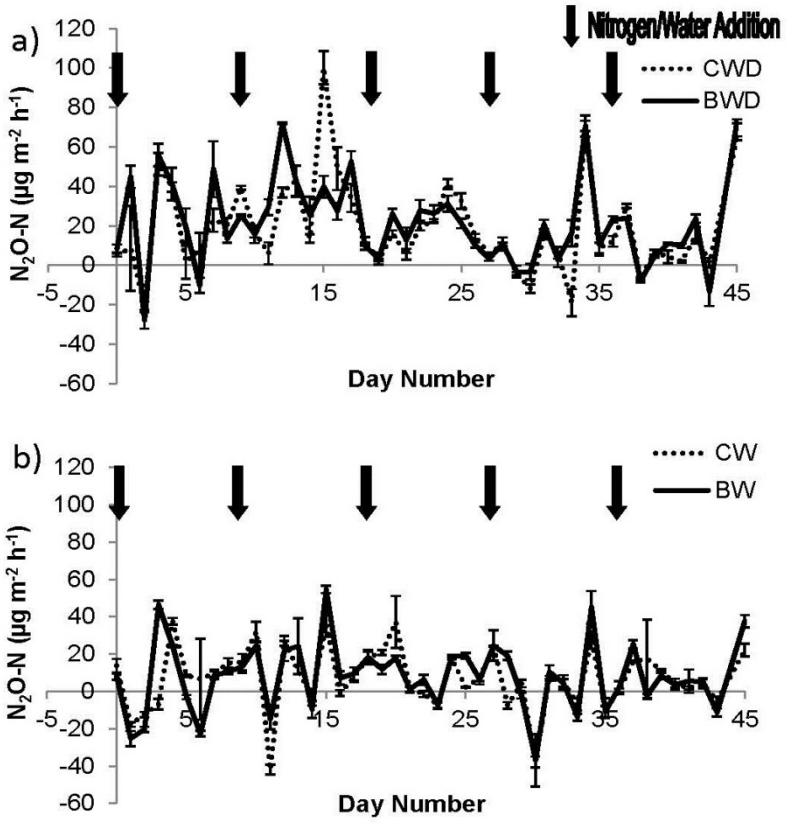
Treatment effects on cumulative N_2O and day 45 results of soil N, C, NO_3^-, NH_4^+, and pH were assessed using two-way analysis of variance (ANOVA). All parameters were log-transformed prior to statistical analysis. Moisture regime, biochar addition, and their interaction were included as fixed effects. Post-hoc comparisons were performed using Tukey's correction for multiple testing. Model fit was assessed using graphical analysis of residuals, a Shapiro-Wilk test for normality, and the O'Brien test for equal variance. The 0.05 confidence interval was used to determine statistical significance. All statistical analyses were conducted using JMP 9.0.0 (SAS Institute, Cary, NC, USA).

Results

Impact of biochar amendment on N_2O fluxes

N_2O emissions typically peaked between N additions with highest treatment mean rates of emission for CWD, BWD, BW and CW columns at approx. 100, 72.9, 54.6, and 37.6 µg N_2O-N m^{-2} h^{-1}, respectively (Figure 1). N_2O emissions were significantly affected by moisture regime (p = 0.0001), but were not affected by presence of biochar (p = 0.5538), and there was no interaction effect (p = 0.9847). Linearity checks indicated that the concentration increases of N_2O released were linear during the incubation period.

Figure 1. Effect of biochar on N_2O emissions from soil that has been subjected to five wetting and drying cycles (a) or that has been kept saturated for 45 days (b), means and standard errors (n = 4) (arrows indicate NH_4NO_3 and water addition)



Soil pH, carbon, and nutrients

Soil pH decreased over the duration of the experiment. Average soil pH was significantly increased by biochar addition ($p < 0.0001$) and by wetting and drying cycles ($p < 0.0001$), though there was no interaction ($p = 0.2595$). The pH values of the BWD columns were significantly higher than those of all other columns, and the pH values of both BW and CWD were significantly higher than those of the CW columns (Table 1).

Soil NO_3^- concentration was significantly decreased in biochar-amended columns ($p < 0.0001$) and in columns that remained saturated throughout the experiment ($p < 0.0001$), though there was no interaction effect ($p = 0.3418$). Tukey test results indicate a significant difference between all mean values, with all columns that had undergone wetting and drying cycles at higher concentrations of NO_3^- than all columns that had been kept saturated, and with biochar-amended columns with lower concentrations of NO_3^- than non-biochar amended columns within each moisture regime.

Soil NH_4^+ was also significantly decreased in biochar-amended columns ($p = 0.0014$) and in columns undergoing wetting and drying cycles ($p = 0.0009$), and there was no interaction effect ($p = 0.3797$). Tukey test results indicate that the CW columns exhibit higher concentrations of NH_4^+ than all other treatments. Although the differences between the treatment means are not significant, average NH_4^+ concentrations of saturated columns are higher than those of columns undergoing wetting and drying cycles, and average NH_4^+ concentrations of biochar-amended columns are lower than average concentrations of their un-amended counter-parts.

Soil C and N results were both increased in biochar-amended columns ($p < 0.0001$ and $p = 0.0272$, respectively), but were not affected by moisture regime ($p = 0.2794$ and 0.6842, respectively), and there was no interaction effect ($p = 0.1919$ and 0.1359, respectively). Tukey test results indicate that C content of biochar-amended columns is significantly higher than that of columns that were not amended with biochar.

Table 1. Cumulative N_2O and Day 45 soil analytical results, means and standard errors (n = 4)

	Moisture Regime			
	Wetting/Drying Cycles		Maintained at 100% WHC	
	Control (CWD)	Biochar Amended (BWD)	Control (CW)	Biochar Amended (BW)
Cumulative N_2O-N (mg kg^{-1} soil)	0.22 ± 0.02a†	0.24 ± 0.02a	0.08 ± 0.02b	0.09 ± 0.02b
NO_3-N (mg kg^{-1})	11.3 ± 0.9a	7.4 ± 0.6b	4.9 ± 0.5c	2.7 ± 0.2d
NH_4-N (mg kg^{-1})	191 ± 11b	155 ± 8b	271 ± 31a	192 ± 7b
Total N (mg kg^{-1})	354 ± 14a	379 ± 27a	328 ± 33a	435 ± 28a
Total C (g kg^{-1})	4.1 ± 0.1b	9.7 ± 2.9a	3.9 ± 0.2b	13.4 ± 1.7a
pH	5.29 ± 0.04b	5.62 ± 0.05a	5.03 ± 0.02c	5.26 ± 0.01b

† Different letters indicate significant differences (p < 0.05) between the results of treatment (Tukey's correction for multiple testing, n = 4)